PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 237/28, 417/12, 401/12, 403/12, A61K 31/495 — A1

(11) International Publication Number: **WO 97/34876**

(43) International Publicati n Date: 25 September 1997 (25.09.97)

(21) International Application Number: PCT/GB97/00650

(22) International Filing Date: 11 March 1997 (11.03.97)

(30) Priority Data:
96400533.4 15 March 1996 (15.03.96) EP
(34) Countries for which the regional or international application was filed: AT et al.

(71) Applicants *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB). ZENECA PHARMA S.A. [FR/FR]; "Le Galien", 1, rue des Chauffours, Boîte postale 127, F-95022 Cergy Cédex (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: THOMAS, Andrew, Peter [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). HENNEQUIN, Laurent, François, André [FR/FR]; Zeneca Pharma S.A., Centre de Recherches, Z.A. La Pompelle, Chemin de Vrilly, Boîte postale 1050, F-51689 Reims Cédex 2 (FR).

(74) Agent: MACK, John, Richard; Zeneca Pharmaceuticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

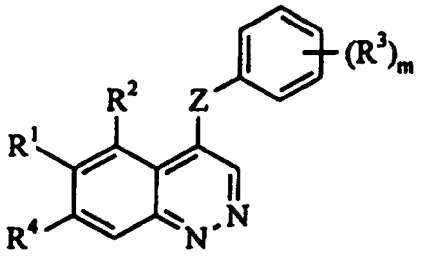
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: CINNOLINE DERIVATIVES AND USE AS MEDICINE

(57) Abstract

The invention relates to the use of cinnoline derivatives of formula (I), wherein Z represents -O-, -NH-, -S- or -CH_2-; m is an integer from 1 to 5; R^1 represents hydrogen, hydroxy, halogeno, nitro, cyano, trifluoromethyl, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio or NR^6R^7 (wherein R^6 and R^7, which may be the same or different, each represents hydrogen or C_{1-3}alkyl); R^2 represents hydrogen, hydroxy, fluoro, chloro, methoxy, amino or nitro; R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro; R^4 represents hydrogen, hydroxy, halogeno, cyano, nitro, amino, trifluoromethyl, C_{1-3}alkyl or a group R^5-X^1 (wherein X^1 represents -O-, -CH_2-, -S-, -SO-, -SO_2-, -NR^8CO-, -$CONR^9$-, -SO_2NR^{10}-, -$NR^{11}SO_2$- or -NR^{12}- (wherein R^8, R^9, R^{10}, R^{11} and R^{12} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxy C_{2-3}alkyl) and R^5 is an optionally substituted alkyl, carbocyclic or heterocyclic group which may be saturated or unsaturated and may be directly linked to the cinnoline ring or be linked via a carbon chain which may have heteroatom linking groups within it and salts thereof, in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being, processes for the preparation of such derivatives, pharmaceutical compositions containing a compound of formula (I) or a pharmaceutically acceptable salt thereof as active ingredient and compounds of formula (I). The compounds of formula (I) and the pharmaceutically acceptable salts thereof inhibit the effects of VEGF, a property of value in the treatment of a number of disease states including cancer and rheumatoid arthritis.



(I)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

CINNOLINE DERIVATIVES AND USE AS MEDICINE.

The present invention relates to the use of cinnoline derivatives in the manufacture of medicaments for use in the production of antiangiogenic and/or vascular permeability reducing effects in warm-blooded animals such as humans, to a method for the treatment of disease states associated with angiogenesis and/or increased vascular permeability, to certain such cinnoline derivatives for use in medicine and to certain cinnoline derivatives per se, processes for their preparation and pharmaceutical compositions containing them as active ingredient.

Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Alteration of vascular permeability is thought to play a role in both normal and pathological physiological processes (Cullinan-Bove et al, 1993, Endocrinology 133: 829-837; Senger et al, 1993, Cancer and Metastasis Reviews, 12: 303-324). Several polypeptides with in vitro endothelial cell growth promoting activity have been identified including, acidic and basic fibroblast growth factors (aFGF & bFGF) and vascular endothelial growth factor (VEGF). By virtue of the restricted expression of its receptors, the growth factor activity of VEGF, in contrast to that of the FGFs, is relatively specific towards endothelial cells. Recent evidence indicates that VEGF is an important stimulator of both normal and pathological angiogenesis (Jakeman et al, 1993, Endocrinology, 133: 848-859; Kolch et al, 1995, Breast Cancer Research and Treatment, 36:139-155) and vascular permeability (Connolly et al, 1989, J. Biol. Chem. 264: 20017-20024). Antagonism of VEGF action by sequestration of VEGF with antibody can result in inhibition of tumour growth (Kim et al, 1993, Nature 362: 841-844).

Receptor tyrosine kinases (RTKs) are important in the transmission of biochemical signals across the plasma membrane of cells. These transmembrane molecules characteristically consist of an extracellular ligand-binding domain connected through a segment in the plasma membrane to an intracellular tyrosine kinase domain. Binding of ligand

to the receptor results in stimulation of the receptor-associated tyrosine kinase activity which leads to phosphorylation of tyrosine residues on both the receptor and other intracellular molecules. These changes in tyrosine phosphorylation initiate a signalling cascade leading to a variety of cellular responses. To date, at least nineteen distinct RTK subfamilies, defined by amino acid sequence homology, have been identified. One of these subfamilies is presently comprised by the fms-like tyrosine kinase receptor, Flt or Flt1, the kinase insert domain-containing receptor, KDR (also referred to as Flk-1), and another fms-like tyrosine kinase receptor, Flt4. Two of these related RTKs, Flt and KDR, have been shown to bind VEGF with high affinity (De Vries et al, 1992, Science 255: 989-991; Terman et al. 1992, Biochem. Biophys. Res. Comm. 1992, 187: 1579-1586). Binding of VEGF to these receptors expressed in heterologous cells has been associated with changes in the tyrosine phosphorylation status of cellular proteins and calcium fluxes.

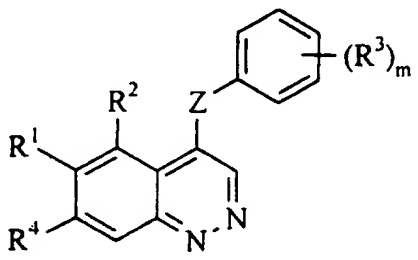
European Patent Publication No. 0326330 discloses certain quinoline, quinazoline and cinnoline plant fungicides. Certain of these plant fungicides are also stated to possess insecticidal and miticidal activity. There is however no disclosure or any suggestion that any of the compounds disclosed may be used for any purpose in animals such as humans. In particular, the European Patent Publication contains no teaching whatsoever concerning angiogenesis and/or increased vascular permeability mediated by growth factors such as VEGF.

A number of further documents described hereinafter disclose certain cinnoline derivatives, but none of these documents contain any teaching whatsoever concerning angiogenesis and/or increased vascular permeabilty mediated by growth factors such as VEGF. Thus J. Med Chem. (1995), 38(18), 3482-7 discloses 4-(3-bromoanilino)cinnoline. J. Chem. Soc. C (1968), (9), 1152-5 discloses 6-chloro-4-phenoxycinnoline. J. Karnatak Univ., Sci. (1984), 29, 82-6 discloses certain 4-anilinocinnolines and Indian J. Chem. (1973), 11(3), 211-13 discloses certain 4-phenylthiocinnolines. All of these disclosed compounds were tested for physiological activity, but all except the two specifically named compounds below were found to be inactive in the tests employed. J. Karnatak Univ., (1973), 18, 25-30 discloses certain 4-phenoxycinnolines some of which showed a little antimicrobial activity but none of which showed analgesic or antihistaminic activity. J. Karnatak Univ., Sci. (1984), 29, 82-6 discloses two compounds: 4-(4-methoxyanilino)-6,7-dimethoxycinnoline and 4-(3-chloroanilino)-6,7-dimethoxycinnoline which were tested and were found to have some anti-inflammatory and

antiarthritic activity. The papers and patents listed below describe the synthesis of certain cinnolines with a phenyl ring linked via a group selected from -O-, -S-, -NH- and -CH_2- at the 4-position: US 5,017,579, US 4,957,925, US 4,994,474, EP 0302793 A2, Arch Pharm (Weinheim) (1970), 303(7), 560-2, J. Med. Chem. (1968), 11, 1270, Indian J. Chem. (1968), 6(7), 350-2, J. Chem. Soc. C (1968), (9), 1156-8, Yakugaku Zasshi (1968), 88(1), 94-7, J. Org. Chem. (1960), 25, 570, J. Org. Chem. (1952), 17, 1571-5, J. Org. Chem. (1953), 18, 1706-8, J. Org. Chem. (1954), 19, 1117-23 and J. Am. Pharm. Assoc. (1959), 48, 135-9, but none of these papers and patents disclose any utility at all for the cinnolines they describe.

The present invention is based on the discovery of compounds that surprisingly inhibit the effects of VEGF, a property of value in the treatment of disease states associated with angiogenesis and/or increased vascular permeability such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. Compounds of the present invention possess good activity against VEGF receptor tyrosine kinase whilst possessing some activity against epidermal growth factor (EGF) receptor tyrosine kinase. Furthermore, compounds of the present invention, possess substantially higher potency against VEGF receptor tyrosine kinase than against EGF receptor tyrosine kinase or FGF R1 receptor tyrosine kinase. Thus compounds of the invention which have been tested possess activity against VEGF receptor tyrosine kinase such that they may be used in an amount sufficient to inhibit VEGF receptor tyrosine kinase whilst demonstrating no significant activity against EGF receptor tyrosine kinase or FGF R1 receptor tyrosine kinase.

According to one aspect of the present invention there is provided the use of a compound of the formula I:



(I)

[wherein:

Z represents -O-, -NH-, -S- or -CH_2-;

m is an integer from 1 to 5;

R^1 represents hydrogen, hydroxy, halogeno, nitro, cyano, trifluoromethyl, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio or NR^6R^7 (wherein R^6 and R^7, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, fluoro, chloro, methoxy, amino or nitro;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

R^4 represents hydrogen, hydroxy, halogeno, cyano, nitro, amino, trifluoromethyl, C_{1-3}alkyl or a group R^5-X^1 (wherein X^1 represents -O-, -CH_2-, -S-, -SO-, -SO_2-, -NR^8CO-, -$CONR^9$-, -SO_2NR^{10}-, -$NR^{11}SO_2$- or -NR^{12}- (wherein R^8, R^9, R^{10}, R^{11} and R^{12} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^5 is selected from one of the following fourteen groups:

1) C_{1-5}alkyl, C_{1-5}hydroxyalkyl, C_{1-5}fluoroalkyl, C_{1-5}aminoalkyl;

2) C_{1-5}alkylX^2COR^{13} (wherein X^2 represents -O- or NR^{14} (in which R^{14} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxyC_{2-3}alkyl) and R^{13} represents C_{1-3}alkyl, $NR^{15}R^{16}$ or OR^{17} (wherein R^{15}, R^{16} and R^{17} which may be the same or different are each hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl), with the proviso that when X^2 is -O-, R^{13} is not OR^{17});

3) C_{1-5}alkylX^3R^{18} (wherein X^3 represents -O-, -S-, -SO-, -SO_2-, -OCO-, -NR^{19}CO-, -$CONR^{20}$-, -SO_2NR^{21}-, -$NR^{22}SO_2$- or -NR^{23}- (wherein R^{19}, R^{20}, R^{21}, R^{22} and R^{23} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{18} represents hydrogen, C_{1-3}alkyl, cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-4}alkoxy and which cyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

4) C_{1-5}alkylX^4C_{1-5}alkylX^5R^{24} (wherein X^4 and X^5 which may be the same or different are each -O-, -S-, -SO-, -SO_2-, -NR^{25}CO-, -$CONR^{26}$-, -SO_2NR^{27}-, -$NR^{28}SO_2$- or -NR^{29}- (wherein R^{25}, R^{26},

R^{27}, R^{28} and R^{29} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{24} represents hydrogen or C_{1-3}alkyl);

5) C_{1-5}alkylR^{30} (wherein R^{30} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

6) C_{2-5}alkenylR^{30} (wherein R^{30} is as defined hereinbefore);

7) C_{2-5}alkynylR^{30} (wherein R^{30} is as defined hereinbefore);

8) $(CH_2)_nR^{31}$ (wherein n is an integer from 0 to 5 and R^{31} is a phenyl group, a pyridone group or a 5 or 6 membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which phenyl, pyridone or aromatic heterocyclic group may carry up to 5 substituents selected from hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}hydroxyalkoxy, carboxy, cyano, $CONR^{32}R^{33}$ and $NR^{34}COR^{35}$ (wherein R^{32}, R^{33}, R^{34} and R^{35}, which may be the same or different, each represents hydrogen, C_{1-4}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

9) C_{2-5}alkenylR^{31} (wherein R^{31} is as defined hereinbefore);

10) C_{2-5}alkynylR^{31} (wherein R^{31} is as defined hereinbefore);

11) C_{1-5}alkylX^6R^{31} (wherein X^6 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{36}CO-$, $-CONR^{37}-$, $-SO_2NR^{38}-$, $-NR^{39}SO_2-$ or $-NR^{40}-$ (wherein R^{36}, R^{37}, R^{38}, R^{39} and R^{40} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{31} is as defined hereinbefore);

12) C_{2-5}alkenylX^7R^{31} (wherein X^7 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{41}CO-$, $-CONR^{42}-$, $-SO_2NR^{43}-$, $-NR^{44}SO_2-$ or $-NR^{45}-$ (wherein R^{41}, R^{42}, R^{43}, R^{44} and R^{45} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{31} is as defined hereinbefore);

13) C_{2-5}alkynylX^8R^{31} (wherein X^8 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{46}CO-$, $-CONR^{47}-$, $-SO_2NR^{48}-$, $-NR^{49}SO_2-$ or $-NR^{50}-$ (wherein R^{46}, R^{47}, R^{48}, R^{49} and R^{50} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{31} is as defined hereinbefore);

14) C_{1-3}alkylX^9C_{1-3}alkylR^{31} (wherein X^9 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{51}CO-$, $-CONR^{52}-$, $-SO_2NR^{53}-$, $-NR^{54}SO_2-$ or $-NR^{55}-$ (wherein R^{51}, R^{52}, R^{53}, R^{54} and R^{55} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{31} is as defined hereinbefore))];

and salts thereof, in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

According to a further aspect of the present invention there is provided a method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I, as defined hereinbefore, or a pharmaceutically acceptable salt thereof.

Z is advantageously -S-, preferably -O-, but especially -NH-.

m is advantageously an integer from 2 to 5, preferably 2 or 3, especially 3.

R^1 is advantageously hydrogen, hydroxy, C_{1-3}alkyl, C_{1-3}alkoxy, amino, cyano, nitro or trifluoromethyl.

R^1 is preferably hydrogen, hydroxy, methyl, ethyl, methoxy or ethoxy, more preferably hydrogen, hydroxy, methyl or methoxy but especially methoxy.

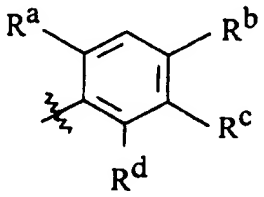
R^2 is advantageously hydrogen, hydroxy, fluoro, methoxy, amino or nitro.

R^2 is preferably hydrogen, amino or nitro, but especially hydrogen.

In one embodiment of the present invention R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, trifluoromethyl, cyano, amino or nitro.

Advantageously in another embodiment of the present invention one R^3 substituent is meta-hydroxy and the other one or more are each selected from halogeno, methyl and methoxy.

In another embodiment of the invention the phenyl group bearing $(R^3)_m$ is preferably of the formula II:



(II)

wherein:

R^a represents hydrogen, methyl, fluoro or chloro, preferably hydrogen, fluoro or chloro, especially fluoro;

R^b represents hydrogen, methyl, methoxy, bromo, fluoro or chloro;

R^c represents hydrogen or hydroxy, especially hydroxy;

R^d represents hydrogen, fluoro or chloro, more preferably hydrogen or fluoro, especially fluoro.

Preferably in another embodiment of the invention two R^3 substituents are halogeno, especially ortho, ortho'- difluoro, and the other one or more are each selected from halogeno, hydroxy and methyl, more preferably from halogeno and methyl.

More preferably in another embodiment of the invention the phenyl group bearing $(R^3)_m$ carries at least two substituents of which one is fluoro, especially ortho-fluoro.

In a particular aspect of the present invention, the phenyl group bearing $(R^3)_m$ is the 2-fluoro-5-hydroxy-4-methylphenyl group, the 4-chloro-2-fluoro-5-hydroxyphenyl group, the 4-bromo-2,6-difluorophenyl group, the 4-chloro-2,6-difluorophenyl group, the 4-chloro-2-fluorophenyl group, the 4-chloro-3-hydroxyphenyl group, the 4-bromo-3-hydroxyphenyl group, the 3-hydroxy-4-methyl group or the 4-bromo-2-fluoro-5-hydroxyphenyl group.

In a particularly preferred aspect of the invention the phenyl group bearing $(R^3)_m$ is the 2-fluoro-5-hydroxy-4-methylphenyl group, the 4-chloro-2-fluoro-5-hydroxyphenyl group, the 4-chloro-3-hydroxyphenyl group, the 4-bromo-3-hydroxyphenyl group, the 3-hydroxy-4-methyl group or the 4-bromo-2-fluoro-5-hydroxyphenyl group.

Conveniently X^1 represents -O-, -S-, $-CH_2-$, $-NR^8CO-$, $-CONR^9-$, $-NR^{11}SO_2-$ or $-NR^{12}-$ (wherein R^8, R^9, R^{11} and R^{12} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Advantageously X^1 represents -O-, -S-, $-NR^8CO-$, $-NR^{11}SO_2-$ or $-NR^{12}-$ (wherein R^8, R^{11} and R^{12} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^1 represents -O-, -S-, $-NR^8CO-$ (wherein R^8 represents hydrogen or methyl) or NH.

More preferably X^1 represents -O- or -NHCO-, especially -O-.

Advantageously X^2 represents -O- or $-NR^{14}-$ (wherein R^{14} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl).

Advantageously X^3 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{19}CO-$, $-NR^{22}SO_2-$ or $-NR^{23}-$ (wherein R^{19}, R^{22} and R^{23} each independently represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl).

Preferably X^3 represents -O-, -S-, -SO-, $-SO_2-$ or $-NR^{23}-$ (wherein R^{23} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl).

More preferably X^3 represents -O-, -S-, -SO-, $-SO_2-$ or $-NR^{23}-$ (wherein R^{23} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl), especially -O- or $-NR^{23}-$ (wherein R^{23} represents hydrogen or C_{1-2}alkyl).

Advantageously X^4 and X^5 which may be the same or different each represents -O-, -S-, -SO-, $-SO_2-$ or $-NR^{29}-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl).

Preferably X^4 and X^5 which may be the same or different each represents -O-, -S- or $-NR^{29}-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl).

More preferably X^4 and X^5 which may be the same or different each represents -O-, -S- or $-NR^{29}-$ (wherein R^{29} represents hydrogen or C_{1-3}alkyl), especially -O- or -NH-.

Advantageously X^6 represents -O-, -S- or $-NR^{40}-$ (wherein R^{40} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^6 represents -O- or $-NR^{40}-$ (wherein R^{40} represents hydrogen or C_{1-2}alkyl).

Advantageously X^7 represents -O-, -S- or $-NR^{45}-$ (wherein R^{45} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^7 represents -O- or $-NR^{45}-$ (wherein R^{45} represents hydrogen or C_{1-2}alkyl).

Advantageously X^8 represents -O-, -S- or $-NR^{50}-$ (wherein R^{50} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^8 represents -O- or $-NR^{50}-$ (wherein R^{50} represents hydrogen or C_{1-2}alkyl).

Advantageously X^9 represents -O-, -S- or $-NR^{55}-$ (wherein R^{55} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^9 represents -O- or $-NR^{55}-$ (wherein R^{55} represents hydrogen or C_{1-2}alkyl).

Conveniently R^4 is hydroxy, halogeno, nitro, trifluoromethyl, C_{1-3}alkyl, cyano, amino or a group R^5-X^1 (wherein X^1 is as defined hereinbefore and R^5 is selected from one of the following fourteen groups:

1) C_{1-5}alkyl, C_{2-5}hydroxyalkyl, C_{1-5}fluoroalkyl, C_{2-5}aminoalkyl;

2) C_{2-3}alkylX^2COR^{13} (wherein X^2 is as defined hereinbefore and R^{13} represents C_{1-3}alkyl, $NR^{15}R^{16}$ or OR^{17} (wherein R^{15}, R^{16} and R^{17} which may be the same or different are each C_{1-2}alkyl or C_{1-2}alkoxyethyl) with the proviso that when X^2 is -O-, R^{13} is not OR^{17});

3) C_{2-4}alkylX^3R^{18} (wherein X^3 is as defined hereinbefore and R^{18} represents hydrogen, C_{1-3}alkyl, cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which C_{1-3}alkyl group may bear

one or two substituents selected from oxo, hydroxy, halogeno and C_{1-3}alkoxy and which cyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy);

4) C_{2-5}alkylX^4C_{2-3}alkylX^5R^{24} (wherein X^4 and X^5 are as defined hereinbefore and R^{24} represents hydrogen or C_{1-3}alkyl);

5) C_{1-5}alkylR^{56} (wherein R^{56} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to C_{1-5}alkyl through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy) or C_{2-5}alkylR^{57} (wherein R^{57} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to C_{2-5}alkyl through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

6) C_{3-4}alkenylR^{58} (wherein R^{58} represents R^{56} or R^{57} as defined hereinbefore);

7) C_{3-4}alkynylR^{58} (wherein R^{58} represents R^{56} or R^{57} as defined hereinbefore);

8) $(CH_2)_nR^{31}$ (wherein n is an integer from 0 to 4 and R^{31} is a phenyl group, a pyridone group or a 5 or 6 membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which phenyl, pyridone or aromatic heterocyclic group may be substituted as hereinbefore defined, advantageously substituted with up to 2 substituents as hereinbefore defined, more preferably substituted with one substituent selected from the group of substituents as hereinbefore defined);

9) 1-R^{31}prop-1-en-3-yl or 1-R^{31}but-2-en-4-yl (wherein R^{31} is as defined hereinbefore);

10) 1-R^{31}prop-1-yn-3-yl or 1-R^{31}but-2-yn-4-yl (wherein R^{31} is as defined hereinbefore);

11) C_{1-5}alkylX^6R^{31} (wherein X^6 and R^{31} are as defined hereinbefore);

12) 1-($R^{31}X^7$)but-2-en-4-yl (wherein X^7 and R^{31} are as defined hereinbefore);

13) 1-($R^{31}X^8$)but-2-yn-4-yl (wherein X^8 and R^{31} are as defined hereinbefore);

14) C_{2-3}alkylX^9C_{1-2}alkylR^{31} (wherein X^9 and R^{31} are as defined hereinbefore)).

Advantageously R^4 is hydroxy, halogeno, nitro, trifluoromethyl, C_{1-3}alkyl, cyano, amino or a group R^5-X^1 (wherein X^1 is as defined hereinbefore and R^5 is selected from one of the following fourteen groups:

1) C_{1-4}alkyl, C_{2-4}hydroxyalkyl, C_{1-4}fluoroalkyl, C_{2-4}aminoalkyl;

2) C_{2-3}alkylX^2COR^{13} (wherein X^2 is as defined hereinbefore and R^{13} represents C_{1-3}alkyl, $NR^{15}R^{16}$ or OR^{17} (wherein R^{15}, R^{16} and R^{17} which may be the same or different are each C_{1-2}alkyl or C_{1-2}alkoxyethyl) with the proviso that when X^2 is -O-, R^{13} is not OR^{17});

3) C_{2-4}alkylX^3R^{18} (wherein X^3 is as defined hereinbefore and R^{18} represents C_{1-3}alkyl, cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^3 through a carbon atom and which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-2}alkoxy and which cyclopentyl, cyclohexyl, pyrrolidinyl or piperidinyl group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

4) C_{2-3}alkylX^4C_{2-3}alkylX^5R^{24} (wherein X^4 and X^5 are as defined hereinbefore and R^{24} represents hydrogen or C_{1-3}alkyl);

5) C_{1-4}alkylR^{59} (wherein R^{59} is a group selected from pyrrolidinyl, piperazinyl, piperidinyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, which group is linked to C_{1-4}alkyl through a carbon atom and which group may carry one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy) or C_{2-4}alkylR^{60} (wherein R^{60} is a group selected from morpholino, thiomorpholino, pyrrolidin-1-yl, piperazin-1-yl and piperidino which group may carry one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

6) C_{3-4}alkenylR^{61} (wherein R^{61} represents R^{59} or R^{60} as defined hereinbefore);

7) C_{3-4}alkynylR^{61} (wherein R^{61} represents R^{59} or R^{60} as defined hereinbefore);

8) $(CH_2)_nR^{31}$ (wherein n is an integer from 1 to 3 and R^{31} is a pyridone group or a 5 or 6 membered aromatic heterocyclic group with 1 to 2 heteroatoms selected from O, N and S, of which preferably one is N which pyridone or aromatic heterocyclic group may be substituted as hereinbefore defined, preferably substituted with one substituent selected from halogeno, C_{1-2}alkyl, C_{1-2}alkoxy, C_{1-2}hydroxyalkyl, C_{1-2}hydroxyalkoxy, carboxy, cyano, $CONR^{32}R^{33}$ and $NR^{34}COR^{35}$ (wherein R^{32}, R^{33}, R^{34} and R^{35}, which may be the same or different, each represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl));

9) 1-R^{31}but-2-en-4-yl (wherein R^{31} is as defined hereinbefore);

10) 1-R^{31}but-2-yn-4-yl (wherein R^{31} is as defined hereinbefore);

11) C_{1-5}alkylX^6R^{31} (wherein X^6 and R^{31} are as defined hereinbefore);

12) 1-($R^{31}X^7$)but-2-en-4-yl (wherein X^7 and R^{31} are as defined hereinbefore);

13) 1-($R^{31}X^8$)but-2-yn-4-yl (wherein X^8 and R^{31} are as defined hereinbefore);

14) C_{2-3}alkylX^9C_{1-2}alkylR^{31} (wherein X^9 and R^{31} are as defined hereinbefore)).

Preferably R^4 is hydroxy, halogeno, nitro, trifluoromethyl, C_{1-3}alkyl, cyano, amino or a group R^5-X^1 (wherein X^1 is as defined hereinbefore and R^5 is selected from one of the following eight groups:

1) C_{1-3}alkyl, C_{2-3}hydroxyalkyl, C_{1-3}fluoroalkyl, C_{2-3}aminoalkyl;

2) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 3-(carbamoyloxy)propyl;

3) C_{2-3}alkylX^3R^{18} (wherein X^3 is as defined hereinbefore and R^{18} represents C_{1-2}alkyl, cyclopentyl, cyclohexyl, pyrrolidinyl or piperidinyl which group is linked to X^3 through a carbon atom and which C_{1-2}alkyl group may bear one or two substituents selected from hydroxy, halogeno and C_{1-2}alkoxy and which cyclopentyl, cyclohexyl, pyrrolidinyl or piperidinyl group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

4) C_{2-3}alkylX^4C_{2-3}alkylX^5R^{24} (wherein X^4 and X^5 are as defined hereinbefore and R^{24} represents hydrogen or C_{1-2}alkyl);

5) C_{1-2}alkylR^{62} (wherein R^{62} is a group selected from pyrrolidinyl, piperazinyl, piperidinyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, which group is linked to C_{1-2}alkyl through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy) or C_{2-3}alkylR^{63} (wherein R^{63} is a group selected from morpholino, thiomorpholino, piperidino, piperazin-1-yl and pyrrolidin-1-yl which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

6) $(CH_2)_nR^{31}$ (wherein n is an integer from 1 to 3 and R^{31} is selected from a pyridone, pyridyl, imidazolyl, thiazolyl, thienyl, pyridazinyl and triazolyl group preferably from a pyridone, pyridyl, imidazolyl, thiazolyl and triazolyl group, more preferably from a pyridone, pyridyl, imidazolyl and triazolyl group and R^{31} may be substituted with one substituent selected from halogeno, C_{1-2}alkyl, C_{1-2}alkoxy, C_{1-2}hydroxyalkyl, C_{1-2}hydroxyalkoxy, carboxy, cyano,

$CONR^{32}R^{33}$ and $NR^{34}COR^{35}$ (wherein R^{32}, R^{33}, R^{34} and R^{35}, which may be the same or different, each represents hydrogen, C_{1-2}alkyl or C_{1-3}alkoxyC_{2-3}alkyl), more preferably substituted with one substituent selected from halogeno, C_{1-2}alkyl, C_{1-2}alkoxy and cyano, especially substituted with one substituent selected from halogeno, C_{1-2}alkyl and cyano, more especially substituted with one substituent selected from chloro, fluoro, methyl and ethyl);

7) C_{1-5}alkylX^6R^{31} (wherein X^6 and R^{31} are as defined hereinbefore);

8) ethylX^9methylR^{31} (wherein X^9 and R^{31} are as defined hereinbefore)).

More preferably R^4 represents hydroxy, C_{1-3}alkyl, amino, or a group R^5-X^1 (wherein X^1 is as defined hereinbefore and R^5 is methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-methylthiazol-4-ylmethyl, 2-acetamidothiazol-4-ylmethyl, 1-methylimidazol-2-ylmethyl, 2-(imidazol-1-yl)ethyl, 2-(1,2,3-triazol-1-yl)ethyl, 2-(1,2,3-triazol-2-yl)ethyl, 2-(1,2,4-triazol-1-yl)ethyl, 2-(1,2,4-triazol-4-yl)ethyl, 4-pyridylmethyl, 2-(4-pyridyl)ethyl, 3-(4-pyridyl)propyl, 2-(4-pyridyloxy)ethyl, 2-(4-pyridylamino)ethyl, 2-(4-oxo-1,4-dihydro-1-pyridyl)ethyl or 2-thiomorpholinoethyl, 3-thiomorpholinopropyl, 2-(2-methoxyethoxy)ethyl, 2-(4-methylpiperazin-1-yl)ethyl or 3-(4-methylpiperazin-1-yl)propyl).

According to another aspect of the present invention there is provided a compound of the formula I as defined hereinbefore with the proviso that where m is 1, R^3 is meta-hydroxy and with the further proviso that the compound of formula I is not 4-(2,6-dimethylphenoxy)-6,7-dimethoxycinnoline; and salts thereof, for use as a medicament.

As indicated above for a compound of formula I, as defined hereinbefore, and salts thereof, for use as a medicament:

m is advantageously an integer from 2 to 5, preferably 2 or 3, especially 3;

Z is preferably -NH-; and

preferably R^4 represents hydroxy, C_{1-3}alkyl, amino, or a group R^5-X^1 (wherein X^1 is as defined hereinbefore and R^5 is methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-methylthiazol-4-ylmethyl, 2-acetamidothiazol-4-ylmethyl, 1-methylimidazol-2-ylmethyl, 2-(imidazol-1-yl)ethyl, 2-(1,2,3-triazol-1-yl)ethyl, 2-(1,2,3-triazol-2-yl)ethyl, 2-(1,2,4-triazol-1-yl)ethyl, 2-(1,2,4-triazol-4-yl)ethyl, 4-pyridylmethyl, 2-(4-pyridyl)ethyl, 3-(4-pyridyl)propyl, 2-(4-pyridyloxy)ethyl, 2-(4-pyridylamino)ethyl, 2-(4-oxo-1,4-dihydro-1-pyridyl)ethyl or 2-thiomorpholinoethyl, 3-thiomorpholinopropyl, 2-(2-methoxyethoxy)ethyl, 2-(4-methylpiperazin-1-yl)ethyl or 3-(4-methylpiperazin-1-yl)propyl).

According to a further aspect of the present invention there is provided a compound of the formula I as defined hereinbefore with the proviso that where m is 1, R^3 is meta-hydroxy and with the further proviso that the phenyl group bearing $(R^3)_m$ is not 3,4-dimethylphenyl and that when the phenyl group bearing $(R^3)_m$ is 2,5-dichlorophenyl, 3,5-dichlorophenyl, 2,3-dichlorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl, 2,6-dichlorophenyl, 2,6-dimethylphenyl, 2-bromo-4-chlorophenyl, 4-bromo-2-chlorophenyl, 2-bromo-4-methylphenyl, 2-chloro-4-methylphenyl, 2-chloro-4-hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 3,5-dichloro-4-hydroxyphenyl, 2,5-dichloro-4-hydroxyphenyl or 5-chloro-2-methylphenyl, Z is -NH-; and salts thereof.

According to a further aspect of the present invention there is provided a compound of the formula I as defined hereinbefore with the proviso that where m is 1, R^3 is meta-hydroxy and with the further proviso that the phenyl group bearing $(R^3)_m$ is not 3,4-dimethylphenyl and that when the phenyl group bearing $(R^3)_m$ is 2,5-dichlorophenyl, 3,5-dichlorophenyl, 2,3-dichlorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl, 2,6-dichlorophenyl, 2,6-dimethylphenyl, 3,4-dimethoxyphenyl, 2-bromo-4-chlorophenyl, 4-bromo-2-chlorophenyl, 2-bromo-4-methylphenyl, 2-chloro-4-methylphenyl, 2-chloro-4-

hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 3,5-dichloro-4-hydroxyphenyl, 2,5-dichloro-4-hydroxyphenyl or 5-chloro-2-methylphenyl, Z is -NH-; and salts thereof.

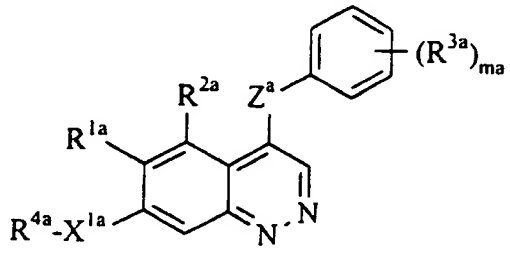
As indicated above for a compound of formula I, as defined hereinbefore, and salts thereof:

m is advantageously an integer from 2 to 5, preferably 2 or 3, especially 3;

Z is preferably-NH-; and

preferably R^4 represents hydroxy, C_{1-3}alkyl, amino, or a group R^5-X^1 (wherein X^1 is as defined hereinbefore and R^5 is methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-methylthiazol-4-ylmethyl, 2-acetamidothiazol-4-ylmethyl, 1-methylimidazol-2-ylmethyl, 2-(imidazol-1-yl)ethyl, 2-(1,2,3-triazol-1-yl)ethyl, 2-(1,2,3-triazol-2-yl)ethyl, 2-(1,2,4-triazol-1-yl)ethyl, 2-(1,2,4-triazol-4-yl)ethyl, 4-pyridylmethyl, 2-(4-pyridyl)ethyl, 3-(4-pyridyl)propyl, 2-(4-pyridyloxy)ethyl, 2-(4-pyridylamino)ethyl, 2-(4-oxo-1,4-dihydro-1-pyridyl)ethyl or 2-thiomorpholinoethyl, 3-thiomorpholinopropyl, 2-(2-methoxyethoxy)ethyl, 2-(4-methylpiperazin-1-yl)ethyl or 3-(4-methylpiperazin-1-yl)propyl).

In a particular embodiment of the present invention there is provided the use of a compound of the formula Ia:



(Ia)

[(wherein:

Z^a represents -O-, -NH-, -S- or $-CH_2-$;

ma is an integer from 1 to 5;

R^{1a} represents hydrogen, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio or $NR^{5a}R^{6a}$ (wherein R^{5a} and R^{6a}, which may be the same or different, each represents hydrogen or C_{1-3} alkyl);

R^{2a} represents hydrogen, hydroxy, fluoro, methoxy, amino or nitro;

R^{3a} represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

X^{1a} represents -O-, $-CH_2-$, -S- or NR^{7a} (wherein R^{7a} represents hydrogen or C_{1-3} alkyl);

R^{4a} is selected from the following:

1) C_{1-5}alkyl, C_{1-5}hydroxyalkyl, C_{1-5}fluoroalkyl, C_{1-5}aminoalkyl;

2) C_{1-5}alkyl$X^{2a}COR^{9a}$ (wherein X^{2a} represents -O- or NR^{8a} (in which R^{8a} represents hydrogen, C_{1-3}alkyl or C_{1-3} alkoxyC_{2-3}alkyl) and R^{9a} represents $NR^{10a}R^{11a}$ or OR^{12a} (wherein R^{10a}, R^{11a} and R^{12a} which may be the same or different are each hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) with the proviso that when X^{2a} is -O-, R^{9a} is not OR^{12a});

3) C_{1-5}alkyl$X^{3a}R^{14a}$ (wherein X^{3a} represents -O-, -S-, -SO-, $-SO_2-$ or NR^{13a} (wherein R^{13a} is hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{14a} represents C_{1-3}alkyl, cyclopentyl or cyclohexyl);

4) C_{1-5}alkyl$X^{4a}C_{1-5}$alkyl$X^{5a}R^{15a}$ (wherein X^{4a} and X^{5a} which may be the same or different are each -O-, -S-, or NR^{16a} (wherein R^{16a} is hydrogen or C_{1-3}alkyl) and R^{15a} represents hydrogen or C_{1-3}alkyl);

5) C_{1-5}alkylsulphonyl$NR^{17a}R^{18a}$ (wherein R^{17a} and R^{18a} which may be the same or different are each hydrogen, C_{1-3} alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

6) C_{1-5}alkylR^{19a} (wherein R^{19a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy); and

7) $(CH_2)_{na}R^{20a}$ (wherein na is an integer from 0 to 5 and R^{20a} is a phenyl group or a 5 or 6 membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S,

which phenyl or aromatic heterocyclic group may carry up to 5 substituents selected from halogeno, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}hydroxyalkoxy, carboxy, cyano, $CONR^{21a}R^{22a}$ and $NR^{23a}COR^{24a}$ (wherein R^{21a}, R^{22a}, R^{23a} and R^{24a}, which may be the same or different, each represents hydrogen or C_{1-4}alkyl)];

and salts thereof in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

In a particular embodiment of the present invention there is provided a method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal in need of such treatment which comprises administering to said animal an effective amount of a compound of formula Ia, as defined hereinbefore, or a pharmaceutically acceptable salt thereof.

Z^a is advantageously -S-, preferably -O-, but especially -NH-.

ma is advantageously an integer from 2 to 5, preferably 2 or 3, especially 3.

R^{1a} is advantageously hydrogen, hydroxy, C_{1-3}alkyl, C_{1-3}alkoxy or amino.

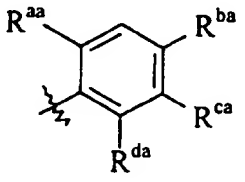
R^{1a} is preferably hydrogen, hydroxy, methyl, ethyl, methoxy or ethoxy, more preferably hydrogen, hydroxy, methyl or methoxy but especially methoxy.

R^{2a} is preferably hydrogen, amino or nitro, but especially hydrogen.

In one embodiment of the present invention R^{3a} represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, trifluoromethyl, cyano, amino or nitro.

Advantageously in another embodiment of the present invention one R^{3a} substituent is meta-hydroxy and the other one or more are each selected from halogeno, methyl and methoxy.

In another embodiment of the invention the phenyl group bearing $(R^{3a})_{ma}$ is preferably of the formula IIaa:



(IIaa)

wherein:

R^{aa} represents hydrogen, methyl, fluoro or chloro, preferably hydrogen, fluoro or chloro, especially fluoro;

R^{ba} represents hydrogen, methyl, methoxy, bromo, fluoro or chloro;

R^{ca} represents hydrogen or hydroxy, especially hydroxy;

R^{da} represents hydrogen, fluoro or chloro, especially fluoro.

Preferably in another embodiment of the invention two R^{3a} substituents are halogeno, especially ortho, ortho'- difluoro, and the other one or more are each selected from halogeno and methyl.

In a particular aspect of the present invention, the phenyl group bearing $(R^{3a})_{ma}$ is the 2-fluoro-5-hydroxy-4-methylphenyl group, the 4-chloro-2-fluoro-5-hydroxyphenyl group, the 4-bromo-2,6-difluorophenyl group, the 4-chloro-2,6-difluorophenyl group or the 4-chloro-2-fluorophenyl group.

X^{1a} is preferably -O-.

Conveniently R^{4a} is selected from one of the following seven groups:

1) C_{1-5}alkyl, C_{2-5}hydroxyalkyl, C_{1-5}fluoroalkyl, C_{2-5}aminoalkyl;

2) C_{2-3}alkylX^{2a}COR^{9a} (wherein X^{2a} represents -O- or NR^{8a} (wherein R^{8a} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl) and R^{9a} represents $NR^{10a}R^{11a}$ or OR^{12a} (wherein R^{10a}, R^{11a} and R^{12a} which may be the same or different are each C_{1-2}alkyl or C_{1-2}alkoxyethyl) with the proviso that when X^{2a} is -O-, R^{9a} is not OR^{12a});

3) C_{2-4}alkyl$X^{3a}R^{14a}$ (wherein X^{3a} represents -O-, -S-, -SO-, -SO_2- or NR^{13a} (wherein R^{13a} is hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl) and R^{14a} represents C_{1-3}alkyl, cyclopentyl or cyclohexyl);

4) C_{2-3}alkyl$X^{4a}C_{2-3}$alkyl$X^{5a}R^{15a}$ (wherein X^{4a} and X^{5a} which may be the same or different are each -O-, -S-, or NR^{16a} (wherein R^{16a} is hydrogen or C_{1-3}alkyl) and R^{15a} represents hydrogen or C_{1-3}alkyl);

5) C_{1-4}alkylsulphonyl$NR^{17a}R^{18a}$ (wherein R^{17a} and R^{18a} which may be the same or different are each hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl);

6) C_{1-5}alkylR^{25a} (wherein R^{25a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to C_{1-5}alkyl through a carbon atom and which heterocyclic group may bear one or two substituents selected from halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy) or C_{2-}

$_{2-5}$alkylR^{26a} (wherein R^{26a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to C_{2-5}alkyl through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy); and

7) $(CH_2)_{na}R^{20a}$ (wherein na is an integer from 0 to 4 and R^{20a} is a phenyl group or a 5 or 6 membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, of which preferably one is N, which phenyl or aromatic heterocyclic group may be substituted as hereinbefore defined, advantageously substituted with up to 2 substituents as hereinbefore defined, more preferably substituted with one substituent selected from the group of substituents as hereinbefore defined).

Advantageously R^{4a} is selected from one of the following seven groups:

1) C_{1-4}alkyl, C_{2-4}hydroxyalkyl, C_{1-4}fluoroalkyl, C_{2-4}aminoalkyl;

2) C_{2-3}alkylX^{2a}COR^{9a} (wherein X^{2a} represents -O- or NR^{8a} (wherein R^{8a} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl) and R^{9a} represents $NR^{10a}R^{11a}$ or OR^{12a} (wherein R^{10a}, R^{11a} and R^{12a} which may be the same or different are each C_{1-2}alkyl or C_{1-2}alkoxyethyl) with the proviso that when X^{2a} is -O-, R^{9a} is not OR^{12a});

3) C_{2-4}alkyl$X^{3a}R^{14a}$ (wherein X^{3a} represents -O-, -S-, -SO-, $-SO_2-$ or NR^{13a} (wherein R^{13a} is hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl) and R^{14a} represents C_{1-3}alkyl, cyclopentyl or cyclohexyl);

4) C_{2-3}alkyl$X^{4a}C_{2-3}$alkyl$X^{5a}R^{15a}$ (wherein X^{4a} and X^{5a} which may be the same or different are each -O-, -S-, or NR^{16a} (wherein R^{16a} is hydrogen or C_{1-3}alkyl) and R^{15a} represents hydrogen or C_{1-3}alkyl);

5) C_{1-4}alkylsulphonyl$NR^{17a}R^{18a}$ (wherein R^{17a} and R^{18a} which may be the same or different are each hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl);

6) C_{1-4}alkylR^{25a} (wherein R^{25a} is selected from pyrrolidinyl, piperazinyl, piperidyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, such that R^{25a} is linked to C_{1-4}alkyl through a carbon atom) or C_{2-4}alkylR^{26a} (wherein R^{26a} is selected from morpholino, pyrrolidin-1-yl, piperazin-1-yl and piperidino); and

7) $(CH_2)_{na}R^{20a}$ (wherein na is an integer from 1 to 3 and R^{20a} is a 5 or 6 membered aromatic heterocyclic group with 1 to 2 heteroatoms selected from O, N and S, of which preferably one

is N, which aromatic heterocyclic group may be substituted as hereinbefore defined, preferably substituted with one substituent selected from halogeno, C_{1-2}alkyl, C_{1-2}alkoxy, C_{1-2}hydroxyalkyl, C_{1-2}hydroxyalkoxy, carboxy, cyano, $CONR^{21a}R^{22a}$ and $NR^{23a}COR^{24a}$ (wherein R^{21a}, R^{22a}, R^{23a} and R^{24a}, which may be the same or different, each represents hydrogen or C_{1-2}alkyl)).

Preferably R^{4a} is selected from one of the following seven groups:

1) C_{1-3}alkyl, C_{2-3}hydroxyalkyl, C_{1-3}fluoroalkyl, C_{2-3}aminoalkyl;

2) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 3-(carbamoyloxy)propyl;

3) C_{2-3}alkyl$X^{3a}R^{14a}$ (wherein X^{3a} represents -O-, -S-, -SO-, $-SO_2-$ or NR^{13a} (wherein R^{13a} is hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl) and R^{14a} represents C_{1-2}alkyl, cyclopentyl or cyclohexyl);

4) C_{2-3}alkyl$X^{4a}C_{2-3}$alkyl$X^{5a}R^{15a}$ (wherein X^{4a} and X^{5a} which may be the same or different are each -O-, or -NH- and R^{15a} represents hydrogen or C_{1-2}alkyl);

5) C_{1-3}alkylsulphonyl$NR^{17a}R^{18a}$ (wherein R^{17a} and R^{18a} which may be the same or different are each hydrogen or methyl);

6) C_{1-2}alkylR^{25a} (wherein R^{25a} is selected from pyrrolidinyl, piperazinyl, piperidyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, such that R^{25a} is linked to C_{1-2}alkyl through a carbon atom) or C_{2-3}alkylR^{26a} (wherein R^{26a} is selected from morpholino, piperidino, piperazin-1-yl and pyrrolidin-1-yl); and

7) $(CH_2)_{na}R^{20a}$ (wherein na is an integer from 1 to 3 and R^{20a} is selected from pyridyl, imidazolyl, thiazolyl, thienyl and pyridazinyl, preferably from pyridyl, imidazolyl and thiazolyl and R^{20a} may be substituted with one substituent selected from halogeno, C_{1-2}alkyl, C_{1-2}alkoxy, C_{1-2}hydroxyalkyl, C_{1-2}hydroxyalkoxy, carboxy, cyano, $CONR^{21a}R^{22a}$ and $NR^{23a}COR^{24a}$ (wherein R^{21a}, R^{22a}, R^{23a} and R^{24a}, which may be the same or different, each represents hydrogen or C_{1-2}alkyl), more preferably substituted with one substituent selected from halogeno, C_{1-2}alkyl and cyano, especially substituted with one substituent selected from chloro, fluoro, methyl and ethyl).

More preferably R^{4a} represents methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl,

2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, 2-(1,3-dioxolan-2-yl)methyl, 3-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-methylthiazol-4-ylmethyl, 1-methylimidazol-2-ylmethyl, 4-pyridylmethyl, 2-(4-pyridyl)ethyl or 3-(4-pyridyl)propyl.

In a further embodiment of the present invention there is provided a compound of the formula Ia as defined hereinbefore with the proviso that where ma is 1, R^{3a} is meta-hydroxy and with the further proviso that the compound of formula Ia is not 4-(2,6-dimethylphenoxy)-6,7-dimethoxycinnoline; and salts thereof, for use as a medicament.

As indicated above for a compound of formula Ia, as defined hereinbefore, and salts thereof, for use as a medicament:

ma is advantageously an integer from 2 to 5, preferably 2 or 3, especially 3;

Z^a is preferably -NH-;

X^{1a} is preferably -O-; and

preferably R^{4a} represents methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-methylthiazol-4-ylmethyl, 1-methylimidazol-2-ylmethyl, 4-pyridylmethyl, 2-(4-pyridyl)ethyl or 3-(4-pyridyl)propyl.

In a further embodiment of the present invention there is provided a compound of the formula Ia as defined hereinbefore with the proviso that where ma is 1, R^{3a} is meta-hydroxy and with the further proviso that the phenyl group bearing $(R^{3a})_{ma}$ is not 3,4-dimethylphenyl and that when the phenyl group bearing $(R^{3a})_{ma}$ is 2,5-dichlorophenyl, 3,5-

dichlorophenyl, 2,3-dichlorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl, 2,6-dichlorophenyl, 2,6-dimethylphenyl, 2-bromo-4-chlorophenyl, 4-bromo-2-chlorophenyl, 2-bromo-4-methylphenyl, 2-chloro-4-methylphenyl, 2-chloro-4-hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 3,5-dichloro-4-hydroxyphenyl, 2,5-dichloro-4-hydroxyphenyl or 5-chloro-2-methylphenyl, Z^a is -NH-; and salts thereof.

In a further embodiment of the present invention there is provided a compound of the formula Ia as defined hereinbefore with the proviso that where ma is 1, R^{3a} is meta-hydroxy and with the further proviso that the phenyl group bearing $(R^{3a})_{ma}$ is not 3,4-dimethylphenyl and that when the phenyl group bearing $(R^{3a})_{ma}$ is 2,5-dichlorophenyl, 3,5-dichlorophenyl, 2,3-dichlorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl, 2,6-dichlorophenyl, 2,6-dimethylphenyl, 3,4-dimethoxyphenyl, 2-bromo-4-chlorophenyl, 4-bromo-2-chlorophenyl, 2-bromo-4-methylphenyl, 2-chloro-4-methylphenyl, 2-chloro-4-hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 3,5-dichloro-4-hydroxyphenyl, 2,5-dichloro-4-hydroxyphenyl or 5-chloro-2-methylphenyl, Z^a is -NH-; and salts thereof.

As indicated above for a compound of formula Ia, as defined hereinbefore, and salts thereof:

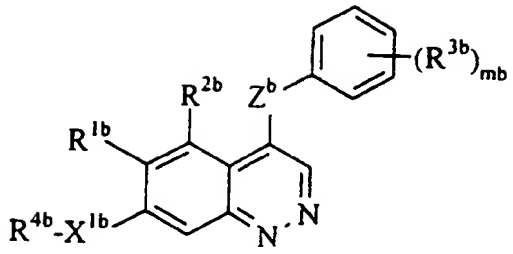
ma is advantageously an integer from 2 to 5, preferably 2 or 3, especially 3;

Z^a is preferably-NH-;

X^{1a} is preferably -O-; and

preferably R^{4a} represents methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-methylthiazol-4-ylmethyl, 1-methylimidazol-2-ylmethyl, 4-pyridylmethyl, 2-(4-pyridyl)ethyl or 3-(4-pyridyl)propyl.

In a further particular embodiment of the current invention there is provided a compound of the formula Ib:



(Ib)

[wherein R^{1b} is hydrogen, C_{1-3}alkoxy, preferably methoxy , or halogeno, preferably chloro;

R^{2b} is hydrogen;

X^{1b} is -O-;

R^{4b} is C_{1-3}alkyl, 2-(C_{1-3}alkoxy)ethyl, benzyl, 4-pyridyl(C_{1-3}alkyl), morpholino(C_{1-3}alkyl), pyrrolidino(C_{1-3}alkyl), 2-methylthiazol-4-yl(C_{1-3}alkyl), 1-methylimidazol-2-yl(C_{1-3}alkyl) and 2-((C_{1-3}alkoxy)(C_{1-3}alkoxy))ethyl;

Z^b is -NH- or -O-;

mb is 2 or 3; and

the phenyl group bearing $(R^{3b})_{mb}$ is selected from: 3-hydroxy-4-methylphenyl, 4-chloro-2-fluorophenyl, 4-bromo-2-fluorophenyl, 4-chloro-2-fluoro-5-hydroxyphenyl, 5-acetoxy-4-chloro-2-fluorophenyl, 2-fluoro-5-hydroxy-4-methylphenyl and 4-bromo-2-fluoro-5-hydroxyphenyl]; and salts thereof.

Particularly preferred compounds of the present invention by virtue of their good activity against VEGF receptor tyrosine kinase activity and their lack of significant activity against epidermal growth factor (EGF) receptor tyrosine kinase include:

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline,

4-(4-bromo-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)cinnoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-[(2-methylthiazol-4-yl)methoxy]cinnoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-[(1-methylimidazol-2-yl)methoxy]cinnoline,

and especially the salts thereof, particularly the hydrochloride salts thereof.

Another particularly preferred compound is 4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline especially the salts thereof. particularly the hydrochloride salts thereof.

Especially preferred compounds are:

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline.

4-(4-bromo-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline.

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline.

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)cinnoline.

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-[(2-methylthiazol-4-yl)methoxy]cinnoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline and especially the salts thereof, particularly the hydrochloride salts thereof.

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by 'hereinbefore defined' or 'defined hereinbefore' the said group encompasses the first occurring and broadest definition as well as each and all of the preferred definitions for that group.

In this specification unless stated otherwise the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. An analogous convention applies to other generic terms. Unless otherwise stated the term "alkyl" advantageously refers to chains with 1-6 carbon atoms, preferably 1-4 carbon atoms. The term "alkoxy" as used herein, unless stated otherwise includes "alkyl"-O- groups in which "alkyl" is as hereinbefore defined. The term "aryl" as used herein unless stated otherwise includes reference to a C_{6-10}aryl group which may, if desired, carry one or more substituents selected from halogeno, alkyl, alkoxy, nitro, trifluoromethyl and cyano, (wherein alkyl and alkoxy are as hereinbefore defined). The term "aryloxy" as used herein unless otherwise stated includes "aryl"-O-groups in which "aryl" is as hereinbefore defined. The term "sulphonyloxy" as used herein refers to alkylsulphonyloxy and arylsulphonyloxy groups in which "alkyl" and "aryl" are as hereinbefore defined. The term "alkanoyl" as used herein unless otherwise stated includes alkylC=O groups in which "alkyl" is as defined hereinbefore, for example ethanoyl refers to $CH_3C{=}O$. In this specification unless

stated otherwise the term "alkenyl" includes both straight and branched chain alkenyl groups but references to individual alkenyl groups such as 2-butenyl are specific for the straight chain version only. Unless otherwise stated the term "alkenyl" advantageously refers to chains with 2-5 carbon atoms, preferably 3-4 carbon atoms. In this specification unless stated otherwise the term "alkynyl" includes both straight and branched chain alkynyl groups but references to individual alkynyl groups such as 2-butynyl are specific for the straight chain version only. Unless otherwise stated the term "alkynyl" advantageously refers to chains with 2-5 carbon atoms, preferably 3-4 carbon atoms.

In formula I, as hereinbefore defined, hydrogen will be present at positions 3 and 8 of the cinnoline group.

Within the present invention it is to be understood that a cinnoline of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which inhibits VEGF receptor tyrosine kinase activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

It is also to be understood that certain cinnolines of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which inhibit VEGF receptor tyrosine kinase activity.

For the avoidance of any doubt, it is to be understood that when X^1 is, for example, a group of formula $-NR^8CO-$, it is the nitrogen atom bearing the R^8 group which is attached to the cinnoline ring and the carbonyl (CO) group is attached to R^5, whereas when X^1 is, for example, a group of formula $-CONR^9-$, it is the carbonyl group which is attached to the cinnoline ring and the nitrogen atom bearing the R^9 group is attached to R^5. A similar convention applies to the other two atom X^1 linking groups such as $-NR^{11}SO_2-$ and $-SO_2NR^{10}-$. When X^1 is $-NR^{12}-$ it is the nitrogen atom bearing the R^{12} group which is linked to the cinnoline ring and to R^5. An analogous convention applies to other groups. It is further to be understood that when X^1 represents $-NR^{12}-$ and R^{12} is $C_{1-3}alkoxyC_{2-3}alkyl$ it is the $C_{2-3}alkyl$ moiety which is linked to the nitrogen atom of X^1 and an analogous convention applies to other groups.

For the avoidance of any doubt, it is to be understood that in a compound of the formula I when R^4 is a group R^5-X^1 and R^5 is, for example, a group of formula $(CH_2)_nR^{31}$, it is the terminal $(CH_2)_n$ moiety which is bound to X^1, similarly when R^5 is, for example, a group of formula C_{2-5}alkenylR^{31} it is the C_{2-5}alkenyl moiety which is bound to X^1 and an analogous convention applies to other groups. When R^5 is a group 1-R^{31}prop-1-en-3-yl it is the first carbon to which the group R^{31} is attached and it is the third carbon which is linked to X^1 and an analogous convention applies to other groups.

The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

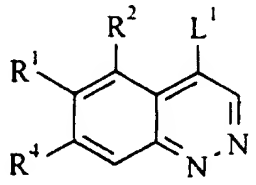
A compound of the formula I, or salt thereof, and other compounds of the invention (as hereinafter defined) may be prepared by any process known to be applicable to the preparation of chemically-related compounds. Such processes include, for example, those illustrated in European Patent Applications Publication Nos. 0520722, 0566226, 0602851 and 0635498. Such processes, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are

obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus the following processes (a) to (g) and (i) to (v) constitute further features of the present invention.

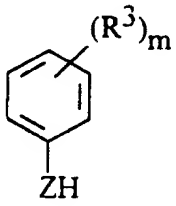
Synthesis of Compounds of Formula I

(a) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula III:



(III)

(wherein R^1, R^2 and R^4 are as defined hereinbefore and L^1 is a displaceable moiety), with a compound of the formula IV:



(IV)

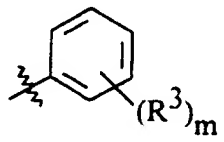
(wherein Z, R^3 and m are as defined hereinbefore) whereby to obtain compounds of the formula I and salts thereof. A convenient displaceable moiety L^1 is, for example, a halogeno, alkoxy (preferably C_{1-4}alkoxy), aryloxy or sulphonyloxy group, for example a chloro, bromo, methoxy, phenoxy, methanesulphonyloxy or toluene-4-sulphonyloxy group.

The reaction is advantageously effected in the presence of either an acid or a base. Such an acid is, for example, an anhydrous inorganic acid such as hydrogen chloride. Such a base is, for example, an organic amine base such as, for example, pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, morpholine, N-methylmorpholine or diazabicyclo[5.4.0]undec-7-ene, or for example, an alkali metal or alkaline earth metal

carbonate or hydroxide, for example sodium carbonate, potassium carbonate, calcium carbonate, sodium hydroxide or potassium hydroxide. Alternatively such a base is, for example, an alkali metal hydride, for example sodium hydride, or an alkali metal or alkaline earth metal amide, for example sodium amide or sodium bis(trimethylsilyl)amide. The reaction is preferably effected in the presence of an inert solvent or diluent, for example an alkanol or ester such as methanol, ethanol, isopropanol or ethyl acetate, a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as tetrahydrofuran or 1,4-dioxan, an aromatic hydrocarbon solvent such as toluene, or a dipolar aprotic solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidin-2-one or dimethylsulphoxide. The reaction is conveniently effected at a temperature in the range, for example, 10 to 150°C, preferably in the range 20 to 80°C.

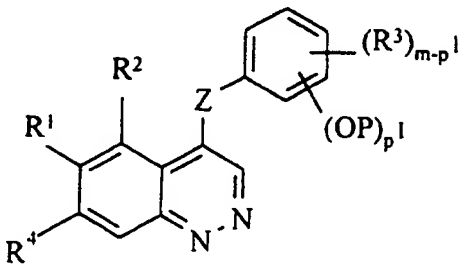
The compound of the invention may be obtained from this process in the form of the free base or alternatively it may be obtained in the form of a salt with the acid of the formula H-L^1 wherein L^1 has the meaning defined hereinbefore. When it is desired to obtain the free base from the salt, the salt may be treated with a base as defined hereinbefore using a conventional procedure.

(b) Where the group of formula IIa:



(IIa)

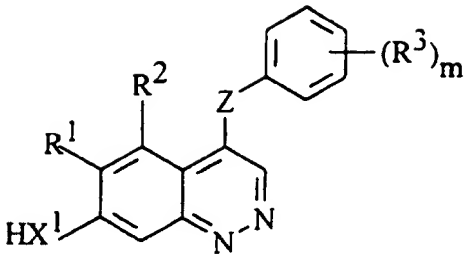
(wherein R^3 and m are as hereinbefore defined) represents a phenyl group carrying one or more hydroxy groups, a compound of the formula I and salts thereof can be prepared by the deprotection of a compound of formula V:



(V)

(wherein m, R^1, R^2, R^3, R^4 and Z are as hereinbefore defined, P represents a phenolic hydroxy protecting group and p^1 is an integer from 1 to 5 equal to the number of protected hydroxy groups and such that m-p^1 is equal to the number of R^3 substituents which are not protected hydroxy). The choice of phenolic hydroxy protecting group P is within the standard knowledge of an organic chemist, for example those included in standard texts such as "Protective Groups in Organic Synthesis" T.W. Greene and R.G.M.Wuts, 2nd Ed. Wiley 1991, including ethers (for example, methyl, methoxymethyl, allyl and benzyl), silyl ethers (for example, t-butyldiphenylsilyl and t-butyldimethylsilyl), esters (for example, acetate and benzoate) and carbonates (for example, methyl and benzyl). The removal of such a phenolic hydroxy protecting group may be effected by any of the procedures known for such a transformation, including those reaction conditions indicated in standard texts such as that indicated hereinbefore, or by a related procedure. The reaction conditions preferably being such that the hydroxy derivative is produced without unwanted reactions at other sites within the starting or product compounds. For example, where the protecting group P is acetate, the transformation may conveniently be effected by treatment of the cinnoline derivative with a base as defined hereinbefore and including ammonia, and its mono and di-alkylated derivatives, preferably in the presence of a protic solvent or co-solvent such as water or an alcohol, for example methanol or ethanol. Such a reaction can be effected in the presence of an additional inert solvent or diluent as defined hereinbefore and at a temperature in the range 0 to 50°C, conveniently at about 20°C.

(c) Production of those compounds of formula I and salts thereof wherein the substituent R^4 represents R^5-X^1 and X^1 is -O-, -S- or -NR^{12}- (wherein R^{12} is as hereinbefore defined) can be achieved by the reaction, conveniently in the presence of a base as defined hereinbefore, of a compound of the formula VI:



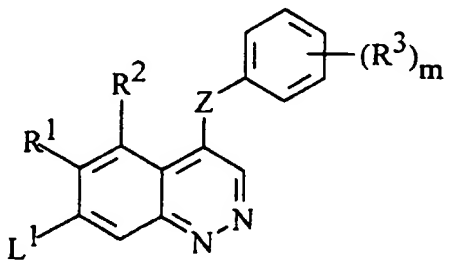
(VI)

(wherein m, X^1, R^1, R^2, R^3, and Z are as hereinbefore defined) with a compound of formula VII:

$$R^5\text{-}L^1 \qquad \text{(VII)}$$

(wherein R^5 and L^1 are as hereinbefore defined); L^1 is a displaceable moiety for example a halogeno or sulphonyloxy group such as a bromo or methanesulphonyloxy group. The reaction is preferably effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 50°C.

(d) Compounds of the formula I and salts thereof wherein the substituent R^4 represents R^5-X^1 may be prepared by the reaction of a compound of the formula VIII:



(VIII)

with a compound of the formula IX:

$$R^5\text{-}X^1\text{-}H \qquad \text{(IX)}$$

(wherein L^1, R^1, R^2, R^3, R^5, Z, m and X^1 are all as hereinbefore defined). The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)). advantageously at a temperature in the range, for example 10 to 150°C. conveniently at about 100°C.

(e) Compounds of the formula I and salts thereof wherein R^4 represents R^5-X^1 and R^5 is C_{1-5}alkylR^{64}, [wherein R^{64} is selected from one of the following four groups:

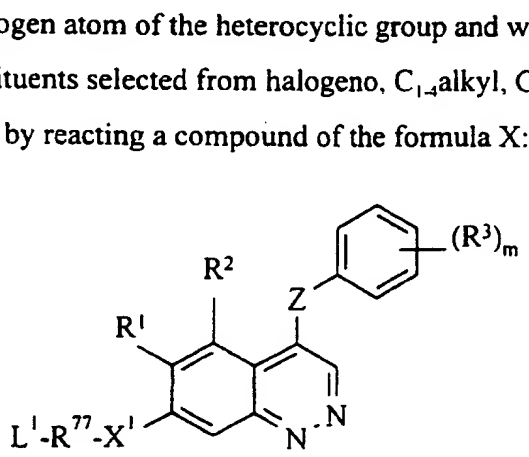
1) $X^{10}C_{1-3}$alkyl (wherein X^{10} represents -O-, -S-, $-SO_2-$, $NR^{65}CO$, $NR^{66}SO_2$ or NR^{67} (wherein R^{65}, R^{66} and R^{67} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

2) $NR^{68}R^{69}$ (wherein R^{68} and R^{69} which may be the same or different are each hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

3) $X^{11}C_{1-5}$alkylX^5R^{24} (wherein X^{11} is -O-, -S-, $-SO_2-$, $NR^{70}CO$, $NR^{71}SO_2$ or NR^{72} (wherein R^{70}, R^{71} and R^{72} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and X^5 and R^{24} are as defined hereinbefore); and

4) an aromatic heterocyclic group selected from pyrrolyl, imidazolyl, pyrazolyl and triazolyl (which aromatic heterocyclic group is linked to the C_{1-5}alkyl moiety via a nitrogen atom of the heterocyclic ring and which aromatic heterocyclic group may carry up to 4 substituents selected from halogeno, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}hydroxyalkoxy, carboxy, cyano, $CONR^{73}R^{74}$ and $NR^{75}COR^{76}$ (wherein R^{73}, R^{74}, R^{75} and R^{76}, which may be the same or different, each represents hydrogen or C_{1-4}alkyl)), or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is nitrogen and the other one may be selected independently from O, S and N, (which heterocyclic group is linked to the C_{1-5}alkyl moiety via a nitrogen atom of the heterocyclic group and which heterocyclic group may bear one or two substituents selected from halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy)],

may be prepared by reacting a compound of the formula X:



(X)

(wherein L^1, X^1, R^1, R^2, R^3, Z and m are as hereinbefore defined and R^{77} is C_{1-3}alkyl) with a compound of the formula XI:

$$R^{64}\text{-}H \qquad (XI)$$

(wherein R^{64} is as defined hereinbefore) to give a compound of the formula I. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), and at a temperature in the range, for example 0 to 150°C, conveniently at about 50°C.

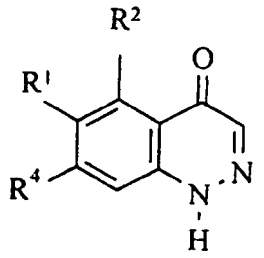
(f) The production of those compounds of the formula I and salts thereof wherein the substituent R^1 is represented by NR^6R^7, where one or both of R^6 and R^7 are C_{1-3}alkyl, may be effected by the reaction of compounds of formula I wherein the substituent R^1 is an amino group and an alkylating agent, preferably in the presence of a base as defined hereinbefore. Such alkylating agents are C_{1-3}alkyl moieties bearing a displaceable moiety as defined hereinbefore such as C_{1-3}alkyl halides for example C_{1-3}alkyl chloride, bromide or iodide. The reaction is preferably effected in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)) and at a temperature in the range, for example, 10 to 100°C, conveniently at about ambient temperature. This process can also be used for preparing compounds in which R^5-X^1 is an alkylamino or dialkylamino group.

(g) The production of compounds of formula I and salts thereof wherein one or more of the substituents R^1, R^2 or R^3 is an amino group or where R^5-X^1 is amino may be effected by the reduction of a corresponding compound of formula I wherein the substituent(s) at the corresponding position(s) of the cinnoline and/or phenyl ring is/are a nitro group(s). The reduction may conveniently be effected as described in process (i) hereinafter. The production of a compound of formula I and salts thereof wherein the substituent(s) at the corresponding position(s) of the cinnoline and/or phenyl ring is/are a nitro group(s) may be effected by the processes described hereinbefore and hereinafter in processes (a-e) and (i-v) using a cinnoline compound selected from the compounds of the formulae (I-XXVIII) in

which the substituent(s) at the corresponding position(s) of the cinnoline and/or phenyl ring is/are a nitro group(s).

Synthesis of Intermediates

(i) The compounds of formula III and salts thereof, constitute a further feature of the present invention. Such compounds in which L^1 is halogeno may for example be prepared by halogenating a compound of the formula XII:

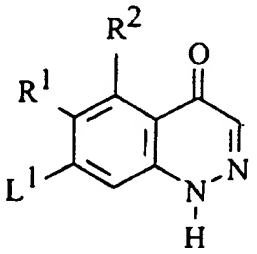


(XII)

(wherein R^1, R^2 and R^4 are as hereinbefore defined).

Convenient halogenating agents include inorganic acid halides, for example thionyl chloride, phosphorus(III)chloride, phosphorus(V)oxychloride and phosphorus(V)chloride. The halogenation reaction is conveniently effected in the presence of an inert solvent or diluent such as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, or an aromatic hydrocarbon solvent such as benzene or toluene. The reaction is conveniently effected at a temperature in the range, for example 10 to 150°C, preferably in the range 40 to 100°C.

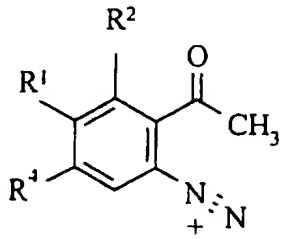
The compounds of formula XII and salts thereof which constitute a further feature of the present invention may for example be prepared by reacting a compound of the formula XIII:



(XIII)

(wherein R^1, R^2 and L^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

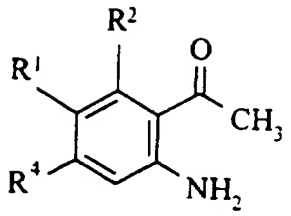
The compounds of formula XII and salts thereof may also be prepared by cyclising a compound of the formula XIV:-



(XIV)

(wherein R^1, R^2 and R^4 are as hereinbefore defined) whereby to form a compound of formula XII or salt thereof. The cyclisation may be conveniently effected in the presence of a mineral or organic acid, for example sulphuric acid, hydrochloric acid or acetic acid or a mixture thereof, preferably at a temperature in the range 20°C to 100°C, especially 50-80°C or if desired under pH-controlled conditions, advantageously at a pH of 4.0 to 8.5. Preferably the pH of the solution is maintained within the range of 6.5 to 8.0. The desired pH is conveniently obtained by the use of an inert base or by the use of an aqueous solution of such a base. Bases which may be used include alkali metal bicarbonates, carbonates or hydroxides or organic amines such as for example pyridine or tertiary amines such as triethylamine, diisopropylethylamine, 2,6-lutidine, collidine, 4-dimethylaminopyridine or methylmorpholine [for example as described in US Patent No. 4,620,000 (L.R. Denes) or DD 258809 (Hirsch et al.]

The compounds of formula XIV and salts thereof, which constitute a further feature of the present invention, may for example be prepared by diazotisation of a compound of the formula XV:-

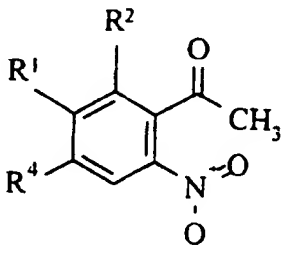


(XV)

(wherein R^1, R^2 and R^4 are as hereinbefore defined). The diazotisation is conveniently effected by the use of an alkali metal nitrite, such as sodium nitrite, in the presence of a mineral acid such as hydrochloric or sulphuric acid or in the presence of an organic acid such as acetic acid or in the presence of a mixture of such acids. The diazotisation is advantageously effected at a temperature in the range between the freezing point of the reaction mixture and 20°C, preferably from 0 to 20°C.

Preferably the compounds of formula XII are prepared by diazotisation and in situ cyclisation of the resulting compound of formula XIV for example as described by Borsch W. and Herbert A. Annalen der Chemie. Volume 546, p293-303.

Compounds of formula XV and salts thereof, which constitute a further feature of the present invention, may for example be prepared by reduction of the nitro group in a compound of formula XVI:

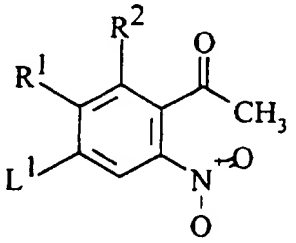


(XVI)

(wherein R^1, R^2 and R^4 are as hereinbefore defined) to yield a compound of formula XV as hereinbefore defined or salt thereof. The reduction of the nitro group may conveniently be effected by any of the procedures known for such a transformation. The reduction may be carried out, for example, by hydrogenation of a solution of the nitro compound in the presence of an inert solvent or diluent as defined hereinbefore preferably in the presence of a metal hydrogenation catalyst such as palladium or platinum. A further reducing agent is, for example, an activated metal such as activated iron (produced for example by washing iron powder with a dilute solution of an acid such as hydrochloric acid). Thus, for example, the reduction may be effected by heating a mixture of the nitro compound and the activated metal in the presence of a solvent or diluent such as a mixture of water and alcohol, for example methanol or ethanol, to a temperature in the range, for example, 50 to 150°C, conveniently at about 70°C.

Where the reduction is effected in the presence of activated iron, this is advantageously produced in situ, conveniently by the use of iron, generally iron powder, in the presence of acetic acid/water and preferably at about 100°C.

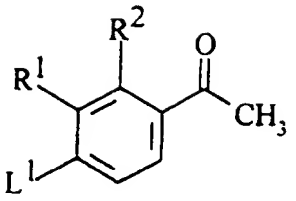
The compounds of formula XVI and salts thereof which constitute a further feature of the present invention may for example be produced by reacting a compound of formula XVII:



(XVII)

(wherein R^1, R^2 and L^1 are as hereinbefore defined) with a compound of formula IX as hereinbefore defined to yield a compound of formula XVI as hereinbefore defined or salt thereof. The reaction of the compounds of formula XVII and IX is conveniently effected under conditions as described for process (d) hereinbefore.

Compounds of formula XVII and salts thereof may for example be prepared by nitration of a compound of the formula XVIII:-



(XVIII)

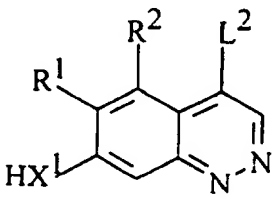
(wherein R^1, R^2 and L^1 are as hereinbefore defined) whereby to form a compound of formula XVIII as hereinbefore defined or a salt thereof. The nitration is conveniently effected in the presence of nitric acid which may be dilute or concentrated, but is preferably about 70% nitric acid. The nitration is conveniently effected at a temperature in the range 0 to 20°C. The nitration may also be effected in the presence of a Lewis acid catalyst such as tin(IV)chloride. Where a Lewis acid catalyst is used the reaction is advantageously effected at a lower temperature, conveniently in the range -50 to 0°C, preferably at about -30°C, preferably in the presence of methylene chloride.

The compounds of formula XVI, as defined hereinbefore, and salts thereof may for example be prepared by nitration of compounds of the formula XVIII in which the L^1 moiety is replaced by R^4. The nitration is conveniently effected as described hereinbefore.

The compounds of formula XIII, as defined hereinbefore, and salts thereof may for example be prepared from compounds of the formulae XIV and XV, in which the R^4 group is replaced by the moiety L^1, the reactions may be effected by processes as described above for the preparation of compounds of formula XII from compounds of formulae XIV and XV.

Compounds of the formula XV in which the R^4 group is replaced by the moiety L^1 may be prepared by the reduction of the nitro group in compounds of the formua XVII, the reduction may be effected as defined hereinbefore.

The compounds of formula III and salts thereof wherein R^4 represents R^5-X^1 may also be prepared for example by reacting a compound of the formula XIX:

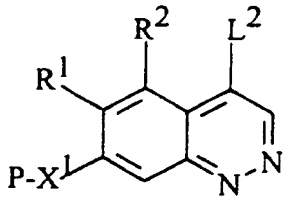


(XIX)

(wherein R^1, R^2 and X^1 are as hereinbefore defined and L^2 represents a displaceable protecting moiety) with a compound of the formula VII as hereinbefore defined whereby to obtain a compound of formula III in which L^1 is represented by L^2.

A compound of formula XIX is conveniently used in which L^2 represents a phenoxy group which may if desired carry up to 5 substituents, preferably up to 2 substituents, selected from halogeno, nitro and cyano. More preferably L^2 is chloro. The reaction may be conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula XIX and salts thereof as hereinbefore defined may for example be prepared by deprotecting a compound of formula XX:

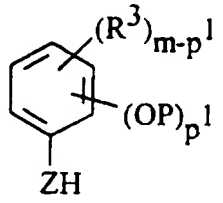


(XX)

(wherein R^1, R^2, P, X^1 and L^2 are as hereinbefore defined). Deprotection may be effected by techniques well known in the literature, for example where P represents a benzyl group deprotection may be effected by hydrogenolysis, or by treatment with trifluoroacetic acid.

One compound of formula III may if desired be converted into another compound of formula III in which the moiety L^1 is different. Thus for example a compound of formula III in which L^1 is other than halogeno, for example optionally substituted phenoxy, may be converted to a compound of formula III in which L^1 is halogeno by hydrolysis of a compound of formula III (in which L^1 is other than halogeno) to yield a compound of formula XII as hereinbefore defined, followed by introduction of halide to the compound of formula XII, thus obtained as hereinbefore defined, to yield a compound of formula III in which L^1 represents halogen.

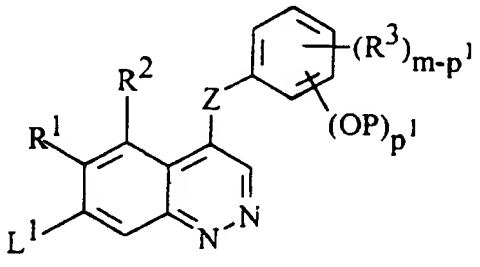
(ii) The compounds of formula V and salts thereof, constitute a further feature of the present invention, and may for example be prepared by the reaction of a compound of formula III as hereinbefore defined with a compound of the formula XXI:



(XXI)

(wherein R^3, m, p^1, P and Z are as hereinbefore defined), whereby to form a compound of formula V as hereinbefore defined or a salt thereof. The reaction may for example be effected as described for process (a) hereinbefore.

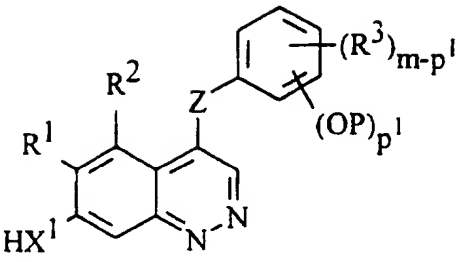
The compounds of formula V and salts thereof wherein R^4 represents R^5-X^1 may also be prepared by reacting a compound of formula XXII:



(XXII)

(wherein R^1, R^2, L^1, Z, R^3, m, p^1 and P are as hereinbefore defined) with a compound of formula IX as hereinbefore defined, whereby to form a compound of formula V as hereinbefore defined or a salt thereof. The reaction may for example be effected as described for process (d) above.

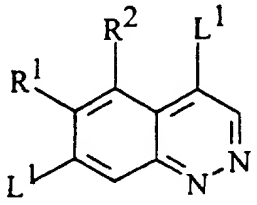
The compounds of formula V and salts thereof wherein R^4 represents R^5-X^1 may also be prepared by reacting a compound of formula XXIII:



(XXIII)

(wherein R^1, R^2, R^3, X^1, Z, P, p^1 and m are as hereinbefore defined) with a compound of the formula VII as hereinbefore defined, whereby to form a compound of formula V as hereinbefore defined or a salt thereof. The reaction may for example be effected as described for process (c) hereinbefore.

The compounds of formula XXII and salts thereof, which constitute a further feature of the present invention, may for example be prepared by reaction of a compound of formula XXIV:



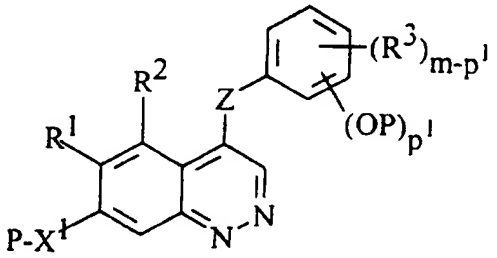
(XXIV)

(wherein R^1, R^2, and L^1 are as hereinbefore defined, and L^1 in the 4- and 7- positions may be the same or different) with a compound of the formula XXI as hereinbefore defined whereby to form a compound of formula XXII as hereinbefore defined or a salt thereof. The reaction may be effected for example by a process as described in (a) above.

Compounds of the formulae XX and XXIV may be prepared by any convenient known method, but may for example be prepared by introducing the moiety L^2 or L^1 as hereinbefore defined into a compound corresponding to a compound of formula XII but in which the group R^4 is replaced by the moiety P-X^1 or L^1. The reaction may be effected for

example by a process as described for the preparation of compounds of formula III from compounds of formula XII as described in (i) above.

Compounds of the formula XXIII and salts thereof, which constitute a further feature of the present invention, may be made by deprotecting a compound of formula XXV:

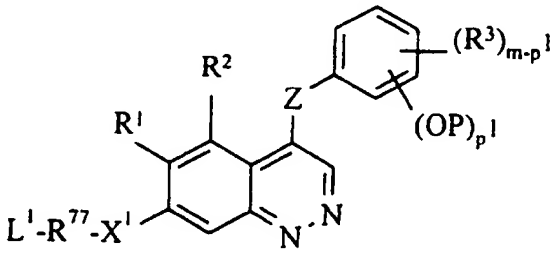


(XXV)

(wherein R^1, R^2, R^3, P, Z, X^1, p^1 and m are as hereinbefore defined) whereby to form a compound of formula XXIII or salt thereof. Deprotection of the compound of formula XXV may for example be effected as described in (i) above.

The compounds of formula XXV and salts thereof constitute a further feature of the present invention and may be prepared, for example, by reacting a compound of formula XX as hereinbefore defined with a compound of formula XXI as hereinbefore defined. The reaction may for example be effected as described in process (a).

Compounds of the formula V, as hereinbefore defined, and salts thereof wherein R^4 represents R^5-X^1 and R^5 is C_{1-5}alkylR^{64}, wherein R^{64} is as hereinbefore defined, may also be prepared by the reaction of a compound of formula XXVI:



(XXVI)

(wherein L^1, R^{77}, X^1, R^1, R^2, R^3, Z, P, m and p^1 are as defined hereinbefore) with a compound of the formula XI as defined hereinbefore, under the conditions described in (e) above.

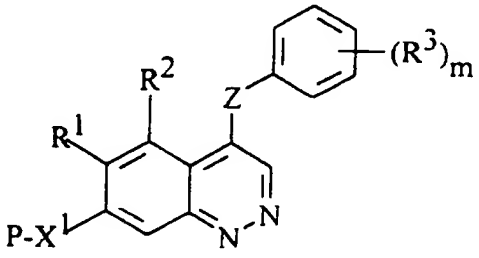
Compounds of the formula XXVI and salts thereof, which constitute a further feature of the present invention, may be made for example by reacting compounds of the formulae XXIII as defined hereinbefore, with a compound of the formula XXVII:

$$L^1\text{-}R^{77}\text{-}L^1$$

(XXVII)

(wherein L^1 and R^{77} are as hereinbefore defined) under the conditions described in (c) above.

(iii) Compounds of the formula VI, as hereinbefore defined, and salts thereof constitute a further feature of the present invention and may be prepared by deprotecting the compound of formula XXVIII:



(XXVIII)

(wherein R^1, R^2, R^3, P, Z, X^1 and m are as hereinbefore defined) for example by a process as described in (i) above.

Compounds of the formula XXVIII and salts thereof, which constitute a further feature of the present invention, may for example be prepared by reacting compounds of the formulae XX and IV as hereinbefore defined, advantageously under the conditions described in (a) hereinbefore, to give a compound of the formula XXVIII as hereinbefore defined or salt thereof.

(iv) Compounds of the formula VIII as hereinbefore defined and salts thereof which constitute a further feature of the present invention may for example be prepared by reacting

compounds of the formulae XXIV and IV as hereinbefore defined, the reaction may for example be effected by a process as described in (a) above.

(v) Compounds of the formula X as defined hereinbefore and salts thereof constitute a further feature of the present invention and may for example be made by reacting compounds of the formulae VI and XXVII as defined hereinbefore, the reaction may be effected for example by a process as described in (c) above.

Compounds of the formula X and salts thereof may also be made for example by deprotecting a compound of the formula XXVI, as hereinbefore defined, by a process for example as described in (b) above.

When a pharmaceutically acceptable salt of a compound of the formula I is required, it may be obtained, for example, by reaction of said compound with, for example, an acid using a conventional procedure, the acid having a pharmaceutically acceptable anion.

Many of the intermediates defined herein are novel, for example, those of the formulae III, V, VI, X, XII, XIV, XV, XVI, XXII, XXIII, XXV, XXVI and XXVIII and these are provided as a further feature of the invention.

The identification of compounds which potently inhibit the tyrosine kinase activity associated with the VEGF receptors such as Flt and/or KDR and which inhibit angiogenesis and/or increased vascular permeability is desirable and is the subject of the present invention. These properties may be assessed, for example, using one or more of the procedures set out below:

(a) In Vitro Receptor Tyrosine Kinase Inhibition Test

This assay determines the ability of a test compound to inhibit tyrosine kinase activity. DNA encoding VEGF or epidermal growth factor (EGF) receptor cytoplasmic domains may be obtained by total gene synthesis (Edwards M, International Biotechnology Lab 5(3), 19-25, 1987) or by cloning. These may then be expressed in a suitable expression system to obtain polypeptide with tyrosine kinase activity. For example VEGF and EGF receptor cytoplasmic domains, which were obtained by expression of recombinant protein in insect cells, were found to display intrinsic tyrosine kinase activity. In the case of the VEGF receptor Flt (Genbank accession number X51602), a 1.7kb DNA fragment encoding most of the cytoplasmic domain, commencing with methionine 783 and including the termination codon, described by Shibuya et al (Oncogene, 1990, 5: 519-524), was isolated from cDNA and cloned

into a baculovirus transplacement vector (for example pAcYM1 (see The Baculovirus Expression System: A Laboratory Guide. L.A. King and R. D. Possee. Chapman and Hall. 1992) or pAc360 or pBlueBacHis (available from Invitrogen Corporation)). This recombinant construct was co-transfected into insect cells (for example Spodoptera frugiperda 21(Sf21)) with viral DNA (eg Pharmingen BaculoGold) to prepare recombinant baculovirus. (Details of the methods for the assembly of recombinant DNA molecules and the preparation and use of recombinant baculovirus can be found in standard texts for example Sambrook et al. 1989. Molecular cloning - A Laboratory Manual. 2nd edition. Cold Spring Harbour Laboratory Press and O'Reilly et al. 1992, Baculovirus Expression Vectors - A Laboratory Manual. W. H. Freeman and Co. New York). For other tyrosine kinases for use in assays. cytoplasmic fragments starting from methionine 806 (KDR. Genbank accession number L04947) and methionine 668 (EGF receptor. Genbank accession number X00588) may be cloned and expressed in a similar manner.

For expression of cFlt tyrosine kinase activity. Sf21 cells were infected with plaque-pure cFlt recombinant virus at a multiplicity of infection of 3 and harvested 48 hours later. Harvested cells were washed with ice cold phosphate buffered saline solution (PBS) (10mM sodium phosphate pH7.4, 138mM NaCl. 2.7mM KCl) then resuspended in ice cold HNTG/PMSF (20mM Hepes pH7.5, 150mM NaCl. 10% v/v glycerol. 1% v/v Triton X100. 1.5mM $MgCl_2$, 1mM ethylene glycol-bis(βaminoethyl ether) N.N.N'.N'-tetraacetic acid (EGTA), 1mM PMSF (phenylmethylsulphonyl fluoride); the PMSF is added just before use from a freshly-prepared 100mM solution in methanol) using 1ml HNTG/PMSF per 10 million cells. The suspension was centrifuged for 10 minutes at 13.000 rpm at 4°C. the supernatant (enzyme stock) was removed and stored in aliquots at -70°C. Each new batch of stock enzyme was titrated in the assay by dilution with enzyme diluent (100mM Hepes pH 7.4. 0.2mM Na_3VO_4, 0.1% v/v Triton X100, 0.2mM dithiothreitol). For a typical batch, stock enzyme is diluted 1 in 2000 with enzyme diluent and 50µl of dilute enzyme is used for each assay well.

A stock of substrate solution was prepared from a random copolymer containing tyrosine. for example Poly (Glu. Ala. Tyr) 6:3:1 (Sigma P3899), stored as 1 mg/ml stock in PBS at -20°C and diluted 1 in 500 with PBS for plate coating.

On the day before the assay 100µl of diluted substrate solution was dispensed into all wells of assay plates (Nunc maxisorp 96-well immunoplates) which were sealed and left overnight at 4°C.

On the day of the assay the substrate solution was discarded and the assay plate wells were washed once with PBST (PBS containing 0.05% v/v Tween 20) and once with 50mM Hepes pH7.4.

Test compounds were diluted with 10% dimethylsulphoxide (DMSO) and 25µl of diluted compound was transferred to wells in the washed assay plates. "Total" control wells contained 10% DMSO instead of compound. Twenty five microlitres of 40mM $MnCl_2$ containing 8µM adenosine-5'-triphosphate (ATP) was added to all test wells except "blank" control wells which contained $MnCl_2$ without ATP. To start the reactions 50µl of freshly diluted enzyme was added to each well and the plates were incubated at room temperature for 20 minutes. The liquid was then discarded and the wells were washed twice with PBST. One hundred microlitres of mouse IgG anti-phosphotyrosine antibody (Upstate Biotechnology Inc. product 05-321), diluted 1 in 6000 with PBST containing 0.5% w/v bovine serum albumin (BSA), was added to each well and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of horse radish peroxidase (HRP)-linked sheep anti-mouse Ig antibody (Amersham product NXA 931), diluted 1 in 500 with PBST containing 0.5% w/v BSA, was added and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) solution, freshly prepared using one 50mg ABTS tablet (Boehringer 1204 521) in 50ml freshly prepared 50mM phosphate-citrate buffer pH5.0 + 0.03% sodium perborate (made with 1 phosphate citrate buffer with sodium perborate (PCSB) capsule (Sigma P4922) per 100ml distilled water), was added to each well. Plates were then incubated for 20-60 minutes at room temperature until the optical density value of the "total" control wells, measured at 405nm using a plate reading spectrophotometer, was approximately 1.0. "Blank" (no ATP) and "total" (no compound) control values were used to determine the dilution range of test compound which gave 50% inhibtion of enzyme activity.

(b) In Vitro HUVEC Proliferation Assay

This assay determines the ability of a test compound to inhibit the growth factor-stimulated proliferation of human umbilical vein endothelial cells (HUVEC).

HUVEC cells were isolated in MCDB 131 (Gibco BRL) + 7.5% v/v foetal calf serum (FCS) and were plated out (at passage 2 to 8), in MCDB 131 + 2% v/v FCS + 3μg/ml heparin – 1μg/ml hydrocortisone, at a concentration of 1000 cells/well in 96 well plates. After a minimum of 4 hours they were dosed with the appropriate growth factor (i.e. VEGF 3ng/ml, EGF 3ng/ml or b-FGF 0.3ng/ml) and compound. The cultures were then incubated for 4 days at 37°C with 7.5% CO_2. On day 4 the cultures were pulsed with 1μCi/well of tritiated-thymidine (Amersham product TRA 61) and incubated for 4 hours. The cells were harvested using a 96-well plate harvester (Tomtek) and then assayed for incorporation of tritium with a Beta plate counter. Incorporation of radioactivity into cells, expressed as cpm, was used to measure inhibition of growth factor-stimulated cell proliferation by compounds.

(c) In Vivo Rat Uterine Oedema Assay

This test measures the capacity of compounds to reduce the acute increase in uterine weight in rats which occurs in the first 4-6 hours following oestrogen stimulation. This early increase in uterine weight has long been known to be due to oedema caused by increased permeability of the uterine vasculature and recently Cullinan-Bove and Koos (Endocrinology, 1993,133:829-837) demonstrated a close temporal relationship with increased expression of VEGF mRNA in the uterus. We have found that prior treatment of the rats with a neutralising monoclonal antibody to VEGF significantly reduces the acute increase in uterine weight, confirming that the increase in weight is substantially mediated by VEGF.

Groups of 20 to 22-day old rats were treated with a single subcutaneous dose of oestradiol benzoate (2.5μg/rat) in a solvent, or solvent only. The latter served as unstimulated controls. Test compounds were orally administered at various times prior to the administration of oestradiol benzoate. Five hours after the administration of oestradiol benzoate the rats were humanely sacrificed and their uteri were dissected, blotted and weighed. The increase in uterine weight in groups treated with test compound and oestradiol benzoate and with oestradiol benzoate alone was compared using a Student T test. Inhibition of the effect of oestradiol benzoate was considered significant when $p<0.05$.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

We have found that compounds of the present invention inhibit VEGF receptor tyrosine kinase activity and are therefore of interest for their antiangiogenic effects and/or their ability to cause a reduction in vascular permeability.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

The antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may cover three main categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, razoxin, thalidomide);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors); and

(iii) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristitine and taxoids like taxol, taxotere); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan).

As stated above the compounds defined in the present invention are of interest for their antiangiogenic and/or vascular permeability reducing effects. Such compounds of the invention are expected to be useful in a wide range of disease states including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin. More particularly such compounds of the invention are expected to inhibit the growth of those primary and recurrent solid tumours which are associated with VEGF, especially those tumours which are significantly dependent on VEGF for their growth and spread, including for example, certain tumours of the colon, breast, prostate, lung, vulva and skin.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of inhibitors of VEGF receptor tyrosine kinase activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:-

[(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon;

(iii) column chromatography (by the flash procedure) and medium pressure liquid chromatography (MPLC) were performed on Merck Kieselgel silica (Art. 9385) or Merck Lichroprep RP-18 (Art. 9303) reversed-phase silica obtained from E. Merck, Darmstadt, Germany;

(iv) yields are given for illustration only and are not necessarily the maximum attainable;

(v) melting points are uncorrected and were determined using a Mettler SP62 automatic melting point apparatus, an oil-bath apparatus or a Koffler hot plate apparatus.

(vi) the structures of the end-products of the formula I were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet;

(vii) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

(viii) petroleum ether refers to that fraction boiling between 40-60°C

(ix) the following abbreviations have been used:-

DMF N,N-dimethylformamide
DMSO dimethylsulphoxide
TFA trifluoroacetic acid.]

Example 1

A solution of 4-chloro-6,7-dimethoxycinnnoline hydrochloride (0.6g, 2.3mmol), 3-hydroxy-4-methylaniline (0.425g, 2.5mmol) and triethylamine (800µl, 6mmol) in DMF (10ml) was refluxed for 1 hour. The solution was cooled and poured directly onto a Diaion (trade mark of Mitsubishi) HP20SS column and elution was performed with acetonitrile/water

(4/6) to give, after evaporation of the solvent, **4-(3-hydroxy-4-methylanilino)-6,7-dimethoxycinnoline** (220mg, 31%) as a cream solid.

m.p. 240-244°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15(s, 3H); 3.97(s, 3H); 3.99(s, 3H); 6.73(d, 1H); 6.85(s, 1H); 7.14(d, 1H); 7.52(s, 1H); 7.69(s, 1H); 8.73(s, 1H); 8.81(s, 1H); 9.6(s, 1H)

MS - ESI: 312 $[MH]^-$

Elemental analysis:	Found	C 62.2	H 5.8	N 13.1
$C_{17}H_{17}N_3O_3$ $0.9H_2O$	Requires	C 62.3	H 5.8	N 12.8%

The starting material, 4-chloro-6,7-dimethoxycinnoline hydrochloride was obtained by heating a solution of 4-hydroxy-6,7-dimethoxycinnoline (1g, 4.8mmol) in thionyl chloride (20ml) containing DMF (2drops) at reflux for 3 hours. After cooling and evaporating the excess thionyl chloride, the solid was triturated with ether and filtered to give 4-chloro-6,7-dimethoxycinnoline hydrochloride (1.2g, quantitative).

The starting material, 4-hydroxy-6,7-dimethoxycinnoline was obtained by adding a solution of sodium nitrite (1.9g, 27mmol) to a solution of 2-amino-4,5-dimethoxyacetophenone (5g, 0.025mol) in acetic acid (90ml) and sulphuric acid (15ml) at a rate to maintain the temperature below 20°C. The mixture was heated at 80°C for 90 minutes. After cooling and concentrating the solution to half its original volume, the residue was poured into ether (800ml). The solid was collected by filtration and suspended in water (200ml). After adjusting to pH7 with sodium hydroxide the solid was filtered, washed with water, methanol and methylene chloride to give 4-hydroxy-6,7-dimethoxycinnoline (4.5g, 87%).

Example 2

A solution of 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (0.4g, 1.5mmol) and 3-hydroxy-4-methylaniline (0.2g, 1.6mmol) in DMF (5ml) was heated at 150°C for 20 minutes. After cooling, isopropanol (15ml) was added and the resulting solid filtered off, washed with isopropanol and dried under vacuum to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline** as the hydrochloride salt (yellow solid, 537mg 91%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.38(s, 3H); 3.55(s, 3H); 3.95(t, 2H); 4.2(s, 3H); 4.5(t, 2H); 7.05(d, 1H); 7.1(s, 1H); 7.4(d, 1H); 7.52(s, 1H); 8.2(s, 1H); 8.55(d, 1H)

MS - ESI: 356 $[MH]^+$

Elemental analysis:	Found	C 58.4	H 5.9	N 10.9
$C_{19}H_{21}N_3O_4$ 1HCl	Requires	C 58.2	H 5.7	N 10.7%

The starting material 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline was obtained by heating a solution of 4-hydroxy-6-methoxy-7-(2-methoxyethoxy)cinnoline (7.8g, 0.031mol) in thionyl chloride (130ml) containing DMF (0.8ml) at 80°C for 2 hours. After dilution with toluene, the mixture was evaporated to dryness. The resulting solid was filtered off, washed with ether, and then dissolved in ethyl acetate. The ethyl acetate solution was washed with saturated aqueous sodium hydrogen carbonate solution and then brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using methylene chloride/ethyl acetate (1/9) as eluent to give 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (6.2g, 74%).

m.p. 171-173°C

The starting material 4-hydroxy-6-methoxy-7-(2-methoxyethoxy)cinnoline was obtained by adding a solution of sodium nitrite (3.9g, 0.056mol) in water (5ml), dropwise, to a solution of 2-amino-4-(2-methoxyethoxy)-5-methoxyacetophenone (12.18g, 0.05mol) in acetic acid (180ml) and sulphuric acid (30ml). After stirring for 90 minutes at 80°C the solution was concentrated to half its original volume and poured into ether (800ml). The solid was collected by filtration and suspended in water (400ml). After adjusting to pH7.6 with 2M aqueous sodium hydroxide solution the resulting solid was filtered off and washed with ether to give 4-hydroxy-6-methoxy-7-(2-methoxyethoxy)cinnoline (8g, 62%).

m.p. 232-234°C

The starting material, 2-amino-4-(2-methoxyethoxy)-5-methoxyacetophenone was obtained by adding iron powder (10g, 0.18mol), in portions, to a solution of 2-nitro-4-(2-methoxyethoxy)-5-methoxyacetophenone (17.3g, 0.064mol) in acetic acid (80ml) heated at 100°C. After stirring for 30 minutes at 100°C, the mixture was cooled and water (20ml) was added. The mixture was extracted with ethyl acetate, the combined extracts were washed with water, saturated sodium carbonate solution and brine and then dried ($MgSO_4$) and the solvent

evaporated. The residue was purified by flash chromatography using methylene chloride/ethyl acetate (8/2 followed by 75/25) as eluent to give 2-amino-4-(2-methoxyethoxy)-5-methoxy-acetophenone (12.52g, 81%).
m.p. 99-101°C

The starting material, 2-nitro-4-(2-methoxyethoxy)-5-methoxyacetophenone, was obtained by adding 3-methoxy-4-(2-methoxyethoxy)acetophenone (18.1g, 0.08mol) in portions over 50 minutes to a solution of 69.5% nitric acid (163ml) cooled to 2°C. After stirring for 2 hours at ambient temperature, the reaction mixture was poured onto ice and extracted with ethyl acetate. The organic layer was washed with water and brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using methylene chloride/ethyl acetate (95/5) as eluent to give 2-nitro-4-(2-methoxyethoxy)-5-methoxyacetophenone (17.4g, 80%) as a pale yellow solid.
m.p. 120-124°C

The starting material, 3-methoxy-4-(2-methoxyethoxy)acetophenone, was obtained by heating a solution of 4-hydroxy-3-methoxyacetophenone (20g, 0.12mol) and, bromomethyl methyl ether (12.4ml, 0.13mol) in DMF (400ml) containing potassium carbonate (49.8g, 0.36mol) at 50°C overnight. After cooling, the reaction mixture was diluted with water and adjusted to pH2. After extraction with ethyl acetate, the organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography using petroleum ether/ethyl acetate (6/4 followed by 1/1) as eluent to give 3-methoxy-4-(2-methoxyethoxy)acetophenone (21.8g, 81%).
m.p. 84-86°C

Example 3

A solution of 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (0.4g, 1.5mmol), (prepared as described for the starting material in Example 2), and 4-chloro-2-fluoroaniline (282µl, 2.5mmol) in DMF (5ml) was treated as described in Example 2, to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline** as the hydrochloride salt (450mg, 72%).
m.p. 279-281°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.35(s, 3H); 3.8(t, 2H); 4.05(s, 3H); 4.4(t, 2H); 7.4(s, 1H); 7.5(d, 1H); 7.7(t, 1H); 7.75(d, 1H); 8.05(s, 1H); 8.3(s, 1H)

MS - ESI: 378 $[MH]^+$

Elemental analysis:	Found	C 52.1	H 4.5	N 10.2
$C_{18}H_{17}N_3O_3ClF$ 1HCl	Requires	C 52.2	H 4.4	N 10.1%

Example 4

4-Chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (0.3g, 1.1mmol), (prepared as described for the starting material in Example 2), was added to a solution of 2,4-dihydroxytoluene (1g, 8mmol) and potassium hydroxide (72mg, 1.3mmol) heated at 150°C. After stirring for 10 minutes at 150°C, the mixture was allowed to cool and then partitioned between ethyl acetate and water. The pH was adjusted to 6 and the organic layer was washed with water and brine and dried ($MgSO_4$) and evaporated. The solid was filtered off, washed with ether and dried under vacuum to give a 1/1 mixture of **4-(3-hydroxy-4-methylphenoxy)-6-methoxy-7-(2-methoxyethoxy)cinnoline** and **4-(3-hydroxy-2-methylphenoxy)-6-methoxy-7-(2-methoxyethoxy)cinnoline** (150mg, 38%).

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 2.05 and 2.1(sx2, 3H); 3.35(s, 3H); 3.8 (t, 2H); 4.1 and 4.15 (sx2, 3H); 4.45(t, 2H); 6.75(m, 1H); 6.85(m, 1H); 7.25(d, 1H); 8.25(d, 1H); 7.7-7.8(m, 2H); 8.52 and 8.65(sx2, 1H)

MS - ESI: 357 $[MH]^+$ 379 $[MNa]^+$

Elemental analysis:	Found	C 64.2	H 5.9	N 7.7
$C_{19}H_{20}N_2O_5$	Requires	C 64.0	H 5.7	N 7.9%

The starting material 2,4-dihydroxytoluene was prepared by adding boron tribromide (3.1ml, 3.2mmol) to a solution of 2,4-dimethoxytoluene (1g, 6.5mmol) in pentane (10ml) at -70°C. The reaction mixture was allowed to warm to ambient temperature and the mixture stirred for a further 2 hours. Ice water and ethyl acetate were then added and the aqueous layer basified to pH9.5 with 2M aqueous sodium hydroxide solution. After stirring for 10 minutes, the organic layer was separated and the aqueous layer extracted with ethyl acetate. The combined organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed

by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/ethyl acetate (9/1) to give 2,4-dihydroxytoluene (759mg, 94%) as a white solid.

Example 5

A solution of 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (0.2g, 0.74mmol), (prepared as described for the starting material in Example 2), and 4-bromo-2-fluoroaniline (155mg, 0.82mmol) in DMF (2.5ml) was heated at 150°C for 45 minutes. After cooling to ambient temperature the mixture was treated as described in Example 3 to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline** as the hydrochloride salt (150mg, 44%).

m.p. 278-281°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.38(s, 3H); 3.85(t, 2H); 4.1(s, 3H); 4.4(t, 2H); 7.45(s, 1H); 7.65(dd, 1H); 7.65(s, 1H); 7.9(d, 1H); 8.1(s, 1H); 8.35(s, 1H)

MS - ESI: 422 $[MH]^+$

Elemental analysis:	Found	C 47.3	H 4.1	N 8.9
$C_{18}H_{17}N_3O_3BrF$ 1HCl	Requires	C 47.1	H 4.0	N 9.2%

Example 6

A solution of 7-benzyloxy-4-chloro-6-methoxycinnoline hydrochloride (3.4g, 10mmol) and 4-chloro-2-fluoro-5-hydroxyaniline, (prepared as described in EP 061741 A2), (1.84g, 11mmol) in DMF (42ml) was heated at 130°C for 20 minutes. The resulting solid was filtered off, washed with isopropanol, ether and dried under vacuum to give **7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxycinnoline** as the hydrochloride salt (yellow solid, 3.5g, 75%).

m.p. 280-284°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.05(s, 3H); 5.4(s, 2H); 7.2(d, 1H); 7.4-7.5(m, 3H); 7.5-7.55(m, 3H); 7.68(d, 1H); 8.2(s, 1H); 8.35(s, 1H)

MS - ESI: 426 $[MH]^+$

Elemental analysis:	Found	C 57.4	H 4.2	N 9.5
$C_{22}H_{17}N_3O_3ClF$ 1HCl	Requires	C 57.2	H 3.9	N 9.1%

The starting material, 7-benzyloxy-4-chloro-6-methoxycinnoline hydrochloride, was obtained by heating a solution of 7-benzyloxy-4-hydroxy-6-methoxycinnoline (11g, 39mmol) in thionyl chloride (180ml) containing DMF (1ml) at reflux for 1 hour. After cooling, excess thionyl chloride was removed by evaporation and azeotroped with toluene. The residue was triturated with ether, filtered off, washed with ether and dried under vacuum to give 7-benzyloxy-4-chloro-6-methoxycinnoline hydrochloride as a cream solid (13.6g, quantitative).

The starting material 7-benzyloxy-4-hydroxy-6-methoxycinnoline was obtained by dropwise addition of a solution of sodium nitrite (4.9g, 0.072mol) in water (10ml) to a solution of 2-amino-4-benzyloxy-5-methoxyacetophenone (16.3g 0.06mol) in acetic acid (250ml) and 70% sulphuric acid (7.3ml). After stirring for 30 minutes, triethylamine (25ml) was added and stirring was continued for 6 hours. After adjusting to pH3.2 with 2M aqueous sodium hydroxide solution, the solid was filtered off, washed with water, ether and dried under vacuum to give 7-benzyloxy-4-hydroxy-6-methoxycinnoline (12.76g, 75%) as a brown solid.

m.p. 262-264°C

The starting material 2-amino-4-benzyloxy-5-methoxyacetophenone was obtained by adding powdered iron (520mg, 9.3mmol) to a solution of 2-nitro-4-benzyloxy-5-methoxyacetophenone (1g, 3.3mmol) in acetic acid (5ml) heated at 100°C. After 30 minutes, the reaction mixture was cooled to ambient temperature and diluted with water. After extraction with ethyl acetate the organic layer was washed with water, brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using petroleum ether/ethyl acetate (3/1) as eluent to give 2-amino-4-benzyloxy-5-methoxyacetophenone (629mg, 70%) as a yellow solid.

m.p. 139-141°C

The starting material 2-nitro-4-benzyloxy-5-methoxyacetophenone was obtained by, addition of a suspension of tin(IV)chloride (15.8ml, 0.13mol) and 69.5% nitric acid (9.1ml, 0.2mol) in methylene chloride (110ml), dropwise over a period of 20 minutes, to a solution of 4-benzyloxy-3-methoxyacetophenone (28.9g, 0.11mol) in methylene chloride (400ml) cooled at -35°C.

After stirring for 20 minutes at -25°C, the mixture was warmed to ambient temperature and poured onto ice/water (1 litre). After extraction with methylene chloride the

organic layer was washed with brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using petroleum ether/ethyl acetate (7/3) as eluent to give 2-nitro-4-benzyloxy-5-methoxyacetophenone (27g, 76%) as a yellow solid.
m.p. 134-136°C

The starting material, 4-benzyloxy-3-methoxyacetophenone, was obtained by heating a solution of 4-hydroxy-3-methoxyacetophenone (20g, 0.12mol), benzyl bromide (15.7ml, 0.13mol) and potassium carbonate (49.8g, 0.36mol) in DMF (400ml) at 40°C overnight. After cooling, the mixture was diluted with water, acidified to approximately pH3 and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using petroleum ether/ethyl acetate (8/2 followed by 65/35) as eluent to give 4-benzyloxy-3-methoxyacetophenone (30.3g, 99%).
m.p. 86-88°C

Example 7

Acetic anhydride (920µl, 9.7mmol) and 4-dimethylaminopyridine (80mg, 0.65mmol) were added to a suspension of 7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxycinnoline (3g, 6.5mmol), (prepared as described in Example 6), in pyridine (50ml). After heating at 110°C for 25 minutes, the solid was filtered off, washed with water and ether and dried under vacuum. The solid was suspended in 2M ethereal hydrogen chloride and the volatiles removed by evaporation to give **4-(5-acetoxy-4-chloro-2-fluoroanilino)-7-benzyloxy-6-methoxycinnoline** as the hydrochloride salt (yellow solid, 3.1g, 94%).
m.p. 240-247°C
^{1}H NMR Spectrum: ($DMSOd_6$) 2.35(s, 3H); 4.05(s, 3H); 5.4(s, 2H); 7.35-7.5(m, 3H); 7.55(s, 1H); 7.55(d, 1H); 7.6(s, 1H); 7.7(d, 1H); 7.95(d, 1H); 8.25(s, 1H); 8.4(s, 1H)
MS - ESI: 468 $[M+H]^+$

Elemental analysis:	Found	C 56.8	H 4.2	N 8.2
$C_{24}H_{19}N_3O_4ClF$ 1HCl	Requires	C 57.2	H 4.0	N 8.3%

Example 8

A solution of 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (0.2g, 0.74mmol), (prepared as described for the starting material in Example 2), and 4-chloro-2-fluoro-5-hydroxyaniline (132mg, 0.82mmol), (prepared as described in EP 061741 A2), in DMF (2.5ml) was heated at 140°C for 45 minutes. The work up procedure was as described in Example 6 and gave **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline** as the hydrochloride salt (yellow solid, 157mg, 49%).

m.p. 296-299°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.4(s, 3H); 3.85(t, 2H); 4.1(s, 3H); 4.4(t, 2H); 7.2(d, 1H); 7.45(s, 1H); 7.65(d, 1H); 8.05(s, 1H); 8.35(d, 1H)

MS - ESI: 394 $[MH]^+$

Elemental analysis:	Found	C 50.1	H 4.4	N 9.8
$C_{18}H_{17}N_3O_4ClF$ 1HCl	Requires	C 50.3	H 4.2	N 9.8%

Example 9

A solution of 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (0.1g, 0.37mmol), (prepared as described for the starting material in Example 2), and 4-bromo-2-fluoro-5-hydroxyaniline (84mg, 0.4mmol), (prepared as described in EP 061741 A2), in DMF (2ml) was heated at 140°C for 1 hour. The work up procedure was as described in Example 6 for the production of the final compound and gave in this example **4-(4-bromo-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline** as the hydrochloride salt (yellow solid, 127mg, 72%).

m.p. 288-289°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.4(s, 3H); 3.85(t, 2H); 4.1(s, 3H); 4.4(t, 2H); 7.2(d, 1H); 7.45(s, 1H); 7.75(d, 1H); 8.1(s, 1H); 8.35(s, 1H)

MS - ESI: 438 $[MH]^+$

Elemental analysis:	Found	C 45.8	H 4.0	N 8.9
$C_{18}H_{17}N_3O_4BrF$ 1HCl	Requires	C 45.5	H 3.8	N 8.9%

Example 10

A suspension of 4-chloro-6-methoxy-7-(4-pyridylmethoxy)cinnoline hydrochloride (0.17g, 0.45mmol) and 4-chloro-2-fluoro-5-hydroxyaniline (102mg, 0.63mmol), (prepared as described in EP 061741 A2), in 2-pentanol (3.5ml) was heated at reflux overnight. After cooling, isopropanol was added. The solid formed was filtered off, washed with isopropanol, ether and dried under vacuum to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(4-pyridylmethoxy)cinnoline** as the hydrochloride salt (yellow solid, 194mg, 86%).

m.p. 243-251°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.12(s, 3H); 5.7(s, 2H); 7.25(d, 1H); 7.55(s, 1H); 7.7(d, 1H); 7.9(d, 2H); 8.3(s, 1H); 8.4(s, 1H); 8.9(d, 2H); 10.85(br s, 1H); 11.55(br s, 1H)

MS - ESI: 427 $[MH]^+$

Elemental analysis:	Found	C 49.6	H 3.9	N 10.8
$C_{21}H_{16}N_4O_3ClF$ 1.3H_2O 1.65HCl	Requires	C 49.4	H 4.0	N 11.0%

The starting material, 4-chloro-6-methoxy-7-(4-pyridylmethoxy)cinnoline hydrochloride, was obtained by adding 4-chloro-7-hydroxy-6-methoxycinnoline (200mg, 0.95mmol), followed by 4-hydroxymethylpyridine (108mg, 1mmol) and 1,1'-(azodicarbonyl)dipiperidine (647mg, 2.5mmol), in portions, to a solution of tri(n-butyl)phosphine (640µl, 2.5mmol) in methylene chloride (6ml). After stirring for 1 hour at ambient temperature, a solution of 7M hydrogen chloride in isopropanol (300µl, 2.1mmol) was added. The resulting solid was filtered off, washed with methylene chloride and ether to give 4-chloro-6-methoxy-7-(4-pyridylmethoxy)cinnoline hydrochloride as a solid (196mg, 55%).

The starting material, 4-chloro-7-hydroxy-6-methoxycinnoline was obtained by heating a solution of 7-benzyloxy-4-chloro-6-methoxycinnoline hydrochloride (3.06g, 9mmol), (prepared as described for the starting material in Example 6), in TFA (30ml) at reflux for 5 hours. After evaporation of the solvent, the residue was suspended in water and adjusted to pH7 with saturated aqueous sodium hydrogen carbonate solution. The resulting solid was filtered off, washed with water and ether and dried under vacuum to give 4-chloro-7-hydroxy-6-methoxycinnoline as a yellow solid (1.78g, 94%).

Example 11

A suspension of 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (0.2g, 0.74mmol), (prepared as described for the starting material in Example 2), and 2-fluoro-5-hydroxy-4-methylaniline (126mg 0.89mmol) in 2-pentanol (2.5ml) was heated at reflux for 7.5 hours. After cooling, isopropanol was added and the solid was filtered off, washed with isopropanol and ether, and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline** as the hydrochloride salt (yellow solid 196mg, 64%).

m.p. 283-290°C

^{1}H NMR Spectrum: (DMSOd_6) 2.2(s, 3H); 3.4(s, 3H); 3.8(t, 2H); 4.1(s, 3H); 4.35(t, 2H); 6.95(d, 1H); 7.25(d, 1H); 7.45(s, 1H); 8.2(s, 1H); 8.22(s, 1H); 9.95(s, 1H); 11.2(br s, 1H)

MS - ESI: 374 $[MH]^+$

Elemental analysis:	Found	C 55.5	H 5.5	N 10.0
$C_{19}H_{20}N_3O_4F$ 0.1H_2O 1HCl	Requires	C 55.4	H 5.2	N 10.2%

The starting material, 2-fluoro-5-hydroxy-4-methylaniline was obtained as follows:

Methyl chloroformate (6.8ml, 88mmol) was added over 30 minutes to a solution of 4-fluoro-2-methylphenol (10g, 79mmol) in 6% aqueous sodium hydroxide solution at 0°C. The mixture was stirred for 2 hours, then extracted with ethyl acetate (100ml). The ethyl acetate extract was washed with water (100ml) and dried ($MgSO_4$) and the solvent removed by evaporation to give 4-fluoro-2-methylphenyl methyl carbonate (11.4g, 78%) as an oil.

^{1}H NMR Spectrum: (DMSOd_6) 2.14(s, 3H), 3.81(s, 3H); 7.05(m, 1H); 7.1-7.5(m, 2H)

A mixture of concentrated nitric acid (6ml) and concentrated sulphuric acid (6ml) was added slowly to a solution of 4-fluoro-2-methylphenyl methyl carbonate (11.34g, 62mmol) in concentrated sulphuric acid (6ml) such that the temperature of the reaction mixture was kept below 50°C. The mixture was stirred for 2 hours, then ice/water was added and the precipitated product collected by filtration. The crude product was purified by chromatography on silica eluting with methylene chloride/hexane progressing through increasingly polar mixtures to methanol/methylene chloride (1/19) to give 4-fluoro-2-methyl-5-nitrophenol (2.5g, 22%) as a solid.

^{1}H NMR Spectrum: (DMSOd_6; CD_3COOD) 2.31(s, 3H); 7.38(d, 1H); 7.58(d, 1H)

MS: 171 $[MH]^+$

A mixture of 4-fluoro-2-methyl-5-nitrophenol (2.1g, 13mmol), iron powder (1g, 18mmol) and iron(II)sulphate (1.5g, 10mmol) in water (40ml) was heated at reflux for 4 hours. The reaction mixture was allowed to cool, neutralised with 2M aqueous sodium hydroxide solution and extracted with ethyl acetate (100ml). The ethyl acetate extract was dried ($MgSO_4$) and the solvent removed by evaporation to give 2-fluoro-5-hydroxy-4-methylaniline (0.8g, 47%) as a solid.

^1H NMR Spectrum: ($DMSOd_6$) 1.94(s, 3H); 4.67(s, 2H); 6.22(d, 1H); 6.65(d, 1H); 8.68(s, 1H)

MS: 142 $[MH]^+$

Example 12

A solution of 4-chloro-6-methoxy-7-(3-morpholinopropoxy)cinnoline hydrochloride (83mg, 0.2mmol) and 4-chloro-2-fluoro-5-hydroxyaniline (42mg, 0.26mmol), (prepared as described in EP 061741 A2), in 2-pentanol (5ml) containing DMF (0.5ml) was heated at 120°C for 2.5 hours. The work up procedure was identical to the synthesis of the final product in Example 11 and gave **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-morpholinopropoxy)cinnoline** as the hydrochloride salt (70mg 65%).

m.p. 218-225°C

^1H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 2.4(m, 2H); 3.35(m, 6H); 3.9(m, 4H); 4.1(s, 3H); 4.4(t, 2H); 7.2(d, 1H); 7.45(s, 1H); 7.6(d, 1H); 8.1(s, 1H); 8.35(s, 1H)

MS - ESI: 463 $[MH]^+$

Elemental analysis:	Found	C 46.1	H 5.2	N 9.5
$C_{22}H_{24}N_4O_4ClF\ 2H_2O\ 1.9HCl$	Requires	C 46.5	H 5.3	N 9.9%

The starting material 4-chloro-6-methoxy-7-(3-morpholinopropoxy)cinnoline hydrochloride was obtained by adding 1-chloro-3-morpholinopropane (190mg, 0.95mmol), (prepared as described in US Patent No. 4004007), to a suspension of 4-chloro-7-hydroxy-6-methoxycinnoline (0.2g, 0.95mmol), (prepared as described for the starting material in Example 10), in DMF (5ml) containing potassium carbonate (327mg, 2.3mmol) and potassium iodide (15mg, 0.095mmol). After heating at 80°C for 2 hours, potassium carbonate (65mg, 0.47mmol) and more 1-chloro-3-morpholinopropane (95mg, 0.47mmol) were added.

After stirring for 4 hours at 80°C. the reaction mixture was cooled and 7M hydrogen chloride in isopropanol (407µl) was added. The solution was poured onto a Diaion (trade mark of Mitsubishi) HP20SS column. using water/methanol (100/0 to 0/100 as a gradient) to give 4-chloro-6- methoxy-7-(3-morpholinopropoxy)cinnoline as an hydrochloride salt (175mg, 44%).

Example 13

A solution of 4-chloro-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline hydrochloride (0.1g, 0.25mmol) and 4-chloro-2-fluoro-5-hydroxyaniline (57mg, 0.35mmol), (prepared as described in EP 061741 A2), in 2-pentanol (5ml) was heated at 120°C for 2.5 hours. The solid was filtered off, washed with isopropanol and then ether and dried under vacuum to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline** as the hydrochloride salt (yellow solid, 94mg, 72%).

m.p. 240-245°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9(m, 2H); 2.1(m, 2H); 2.3(m, 2H); 3.1(m, 2H); 3.35(m, 2H); 3.65(m, 2H); 4.1(s, 3H); 4.4(t, 2H); 7.2(d, 1H); 7.45(s, 1H); 7.65(d, 1H); 8.15(s, 1H); 8.35(s, 1H)

MS - ESI: 447 $[MH]^+$

Elemental analysis:	Found	C 49.9	H 5.2	N 10.5
$C_{22}H_{24}N_4O_3ClF\ 0.5H_2O\ 1.95HCl$	Requires	C 50.1	H 5.2	N 10.6%

The starting material, 4-chloro-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline hydrochloride was obtained by adding 4-chloro-7-hydroxy-6-methoxycinnoline (0.3g, 1.4mmol), (prepared as described for the starting material in Example 10), to a solution of 3-pyrrolidinopropyl chloride (275mg, 1.5mmol), (prepared as described in J. Amer. Chem. Soc. 1955, 77, 2272), in DMF (5ml) containing potassium carbonate (491mg, 3.5mmol) and potassium iodide (24mg, 0.14mmol). After stirring at 80°C for 3 hours potassium carbonate (98mg, 0.7mmol) and 3-pyrrolidinopropyl chloride (137mg, 0.7mmol) were added. After 30 minutes. the mixture was cooled and 7M hydrogen chloride in isopropanol (407µl) was added. The solution was poured onto a Diaion (trade mark of Mitsubishi) HP20SS TM column eluting with water/methanol (100/0 to 0/100 as a gradient). After evaporation of the solvent.

the product was purified by preparative HPLC using a reverse phase C18 column eluting with water/methanol (100/0 to 80/20 as a gradient) to give after concentration, 4-chloro-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline hydrochloride (265mg, 48%) as a yellow solid.

Example 14

A suspension of 4-chloro-7-methoxycinnoline hydrochloride (196mg, 0.85mmol) and 3-hydroxy-4-methylaniline (123mg, 1mmol) in 2-pentanol (5ml) was heated at reflux for 2 hours. After cooling, the solid was filtered off, washed with isopropanol, ether and dried under vacuum to give **4-(3-hydroxy-4-methylanilino)-7-methoxycinnoline** as the hydrochloride salt (yellow solid, 215mg,80%).

m.p. 270-275°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(s, 3H); 4.0(s, 3H); 6.88(d, 1H); 6.93(s, 1H); 7.25(d, 1H); 7.35(s, 1H); 7.52(dd, 1H); 8.4(s, 1H); 8.75(d, 1H); 9.98(s, 1H); 11.65(br s, 1H)

MS - ESI: 281 $[MH]^+$

Elemental analysis:				
$C_{16}H_{15}N_3O_2$ 0.14H_2O 1HCl	Found	C 59.7	H 5.4	N 13.0
	Requires	C 60.0	H 5.1	N 13.1%

The starting material 4-chloro-7-methoxycinnoline hydrochloride was obtained by heating a solution of 4-hydroxy-7-methoxycinnoline (352mg,2mmol), (prepared as described in J. Chem. Soc. 1955, 2100), in thionyl chloride (3.5ml) containing DMF (20µl) at reflux, for 1 hour. After removing excess thionyl chloride by evaporation and azeotroping with toluene. The residue was triturated with ether, filtered off and washed with ether to give 4-chloro-7-methoxycinnoline hydrochloride as a yellow solid (450mg, 97%).

Example 15

A solution of 4-chloro-6-methoxy-7-(3-morpholinopropoxy)cinnoline hydrochloride (132mg, 0.33mmol), (prepared as described for the starting material in Example 12), and 2-fluoro-5-hydroxy-4-methylaniline (56mg, 0.39mmol), (prepared as described for the starting material in Example 11), in 2-pentanol (2.5ml) containing 7M hydrogen chloride in isopropanol (9drops) was heated at reflux for 30 minutes. After cooling, the solid was filtered off, washed with isopropanol, ether and dried under vacuum to give **4-(2-fluoro-5-hydr xy-4-**

methylanilin)-6-methoxy-7-(3-morpholinopropoxy)cinnoline as an hydrochloride salt (yellow solid, 143mg, 84%).

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 2.2(s, 3H); 2.35(m, 2H); 3.3(m, 6H); 3.9(br s, 4H); 4.0(s, 3H); 4.35(t, 2H); 6.95(d, 1H); 7.15(d, 1H); 7.35(s, 1H); 8.1(s, 1H); 8.2(s, 1H)

MS - ESI 443 $[MH]^+$

Elemental analysis:	Found	C 53.0	H 6.0	N 10.2
$C_{23}H_{27}N_4O_4F$ 0.5H_2O 2.8HCl 0.2 isopropanol	Requires	C 53.6	H 6.0	N 10.6%

Example 16

A solution of 4-chloro-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline hydrochloride (158mg, 0.4mmol),(prepared as described for the starting material in Example 13), and 2-fluoro-5-hydroxy-4-methylaniline (67mg, 0.48mmol), (prepared as described for the starting material in Example 11), in 2-pentanol (5ml) was heated at reflux for 1 hour. After cooling, the solid was filtered off, washed with isopropanol, ether and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline** as the hydrochloride salt (yellow solid, 55mg, 27%).

m.p. 247-253°C

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 2.05(m, 4H); 2.25(s, 3H); 2.35(m, 2H); 3.25-3.5(br s, 6H); 4.05(s, 3H); 4.4(br s, 2H); 7.0(d, 1H); 7.22(d, 1H); 7.45(s, 1H); 8.15(s, 1H); 8.25(s, 1H)

MS - ESI: 427 $[MH]^+$

Elemental analysis:	Found	C 53.6	H 6.1	N 10.4
$C_{23}H_{27}N_4O_3F$ 1H_2O 1.95HCl	Requires	C 53.6	H 6.1	N 10.9%

Example 17

A suspension of 4-chloro-6-methoxy-7-[(2-methylthiazol-4-yl)methoxy]cinnoline (150mg, 0.46mmol) and 2-fluoro-5-hydroxy-4-methylaniline (78mg, 0.56mmol), (prepared as described for the starting material in Example 11), in 2-pentanol (3ml) and a 5M solution of hydrogen chloride in isopropanol (105µl) was heated at reflux for 90 minutes. The solid obtained was filtered off, washed with isopropanol followed by ether to give **4-(2-fluoro-5-**

hydroxy-4-methylanilino)-6-meth xy-7-[(2-methylthiaz l-4-yl)meth xy]cinnoline hydrochloride as a pale yellow solid (190mg, 82%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.19(s, 3H); 2.69(s, 3H); 4.03(s, 3H); 5.39(s, 2H); 6.98(d, 1H); 7.24(d, 1H); 7.66(s, 1H); 7.76(s, 1H); 8.16(d, 1H); 8.27(s, 1H); 10.0(br s, 1H); 11.32(s, 1H)

MS - ESI m/z: 427 $[MH]^+$

Elemental analysis:	Found	C 50.7	H 4.4	N 11.0
$C_{21}H_{19}N_4O_3SF$ 0.5H_2O 1.65HCl	Requires	C 50.9	H 4.4	N 11.3%

The starting material 4-chloro-6-methoxy-7-[(2-methylthiazol-4-yl)methoxy]cinnoline was obtained by adding potassium carbonate (786mg, 5.7mmol) followed by 4-chloromethyl-2-methylthiazole (308mg, 2mmol) to a suspension of 4-chloro-7-hydroxy-6-methoxycinnoline (0.4g, 1.9mmol), (prepared as described for the starting material in Example 10), in DMF (10ml). After stirring for 4.5 hours at 60°C, the reaction mixture was diluted with water and acidified to pH4 with 2M hydrochloric acid. After extraction with ethyl acetate, the organic layer was washed with water and then brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using methylene chloride/ethyl acetate (2/8) as eluent to give 4-chloro-6-methoxy-7-[(2-methylthiazol-4-yl)methoxy]cinnoline as a solid (293mg, 48%).

Example 18

A suspension of 4-chloro-6-methoxy-7-[(1-methylimidazol-2-yl)methoxy]cinnoline (109mg, 0.35mmol) and 2-fluoro-5-hydroxy-4-methylaniline (70mg, 0.5mmol), (prepared as described for the starting material in Example 11), in 2-pentanol (3ml), DMF (0.5ml) and a 5M solution of hydrogen chloride in isopropanol (74µl, 0.35mmol) was heated at reflux for 3 hours. The solution was then cooled to 0°C and the resulting solid filtered off and washed with ether to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-[(1-methylimidazol-2-yl)methoxy]cinnoline** hydrochloride as a yellow solid (124mg, 73%).
m.p. 215-221°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.2(s, 3H); 3.9(s, 3H); 4.05(s, 3H); 5.7(s, 2H); 6.99(d, 1H); 7.24(d, 1H); 7.67(s, 1H); 7.73(s, 1H); 7.77(s, 1H); 8.20(d, 1H); 8.39(s, 1H); 9.96(s, 1H); 11.5(s, 1H)

MS - ESI m/z: 410 $[MH]^-$

Elemental analysis:	Found	C 51.3	H 4.9	N 13.7
$C_{21}H_{20}N_5O_3F$ 0.9H_2O 1.8HCl	Requires	C 51.3	H 4.8	N 14.3%

The starting material, 4-chloro-6-methoxy-7-[(1-methylimidazol-2-yl)methoxy]-cinnoline was obtained by adding potassium carbonate (531mg, 3.8mmol) followed by 2-chloromethyl-1-methylimidazole (232mg, 1.4mmol) to a suspension of 4-chloro-7-hydroxy-6-methoxycinnoline (270mg, 1.28mmol), (prepared as described for the starting material in Example 10), in DMF (6ml). After stirring overnight at 40°C the mixture was diluted with water and adjusted to pH7. After extraction with ethyl acetate, the organic layer was washed with water and then brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using methylene chloride/methanol (98/2) as eluent to give 4-chloro-6-methoxy-7-[(1-methylimidazol-2-yl)methoxy]cinnoline (111mg, 29%).

Example 19

A solution of 4-chloro-6-methoxy-7-(4-pyridylmethoxy)cinnoline hydrochloride (268mg, 0.71mmol), (prepared as described for the starting material in Example 10), and 2-fluoro-5-hydroxy-4-methylaniline, (109mg, 0.77mmol), (prepared as described for the starting material in Example 11), in 2-pentanol (6ml) was heated at reflux for 4 hours. After dilution with isopropanol, the solid was filtered off, washed with isopropanol followed by ether to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)cinnoline** hydrochloride (92mg, 29%).

m.p. 244-252°C

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 2.2(s, 3H); 4.1(s, 3H); 5.68(s, 2H); 7.0(s, 1H); 7.25(d, 1H); 7.5(s, 1H); 7.9(s, 2H); 8.25(d, 2H); 8.85(s, 2H)

MS - ESI m/z: 407 $[MH]^-$

Elemental analysis:	Found	C 58.0	H 4.8	N 12.3
$C_{22}H_{19}N_4O_3F$ 0.4H_2O 1.1HCl	Requires	C 58.2	H 4.6	N 12.4%

Example 20

A solution of 4-(5-benzyloxy-2-fluoro-4-methylphenoxy)-6-methoxy-7-(2-methoxyethoxy)cinnoline (242mg, 0.5mmol) in a mixture of methanol (9ml) and DMF (10.5ml) containing 10% palladium-on-charcoal catalyst (100mg) was stirred under hydrogen at 5 atmospheres pressure for 9 hours. The catalyst was removed by filtration and the solvent was evaporated. The residue was washed with methanol and ether and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylphenoxy)-6-methoxy-7-(2-methoxyethoxy)cinnoline** as a white solid (87mg, 44%).

m.p. 267-273°C

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 2.18(s, 3H); 3.37(s, 3H); 3.80(t, 2H); 4.04(s, 3H); 4.39(t, 2H); 6.8(d, 1H); 7.2(d, 1H); 7.49(s, 1H); 7.79(s, 1H); 8.49(s, 1H)

MS - ESI m/z: 375 $[MH]^-$

Elemental analysis:	Found	C 58.9	H 5.3	N 7.5
$C_{19}H_{19}N_2O_5F$ 0.7H_2O 0.06DMF	Requires	C 58.9	H 5.4	N 7.4%

The starting material, 4-(5-benzyloxy-2-fluoro-4-methylphenoxy)-6-methoxy-7-(2-methoxyethoxy)cinnoline was obtained by heating a solution of 5-benzyloxy-2-fluoro-4-methylphenol (314mg, 1.3mmol) and 4-chloro-6-methoxy-7-(2-methoxyethoxy)cinnoline (280mg, 1mmol), (prepared as described for the starting material in Example 2), in pyridine (6ml), at reflux for 15 hours. After evaporation of the solvent, the residue was partitioned between ethyl acetate and water adjusted to pH7. The organic layer was separated, washed with water and then brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using methylene chloride/ether (4/6 followed by 3/7) as eluent to give 4-(5-benzyloxy-2-fluoro-4-methylphenoxy)-6-methoxy-7-(2-methoxyethoxy)cinnoline as a white solid (247mg, 53%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.27(s, 3H); 3.37(s, 3H); 3.8(t, 2H); 4.0(s, 3H); 4.4(t, 2H); 5.14(s, 2H); 7.26(d, 1H); 7.3-7.5(m, 6H); 7.5(s, 1H); 7.81(s, 1H); 8.44(s, 1H)

The starting material 5-benzyloxy-2-fluoro-4-methylphenol, was obtained by adding a solution of sodium nitrite (1.68g, 24mmol) in water (3.5ml), dropwise, to a solution of 5-benzyloxy-2-fluoro-4-methylaniline (4.7g, 20mmol) in acetic acid (82ml) and 70% sulphuric

acid (3.15ml) cooled at 10°C. The mixture was stirred vigorously for 20 minutes. then a solution of copper(II)nitrate trihydrate (481g, 2mol) in water (790ml) was added. followed by copper(II)oxide (3g, 19mmol). After stirring for 3 hours, the mixture was extracted with ethyl acetate. The organic layer was washed with water and then brine. dried ($MgSO_4$) and the solvent evaporated. The resulting oil was purified by flash chromatography using petroleum ether/ether (85/15) as eluent to give 5-benzyloxy-2-fluoro-4-methylphenol as an orange oil (1.25g, 27%).

The starting material, 5-benzyloxy-2-fluoro-4-methylaniline. was obtained by adding iron powder (2.88g, 51mmol), in portions. to a solution of 5-benzyloxy-2-fluoro-4-methylnitrobenzene (4.8g, 18mmol) in acetic acid (33ml) and water (5.7ml), at ambient temperature. The mixture was heated and after stirring at 100°C for 10 minutes. it was cooled and partitioned between ethyl acetate and water. The organic layer was washed with water. aqueous saturated sodium carbonate solution and then brine, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography using petroleum ether/ethyl acetate (8/2) as eluent to give 5-benzyloxy-2-fluoro-4-methylaniline (3.65g, 87%).

The starting material 5-benzyloxy-2-fluoro-4-methylnitrobenzene was obtained by adding benzyl bromide (3ml, 25mmol) to a solution of 2-fluoro-5-hydroxy-4-methylnitrobenzene (3.92mg, 23mmol) in DMF (70ml) containing potassium carbonate (9.5g, 68mmol). After stirring for 3 hours at 60°C. the mixture was diluted with water and adjusted to pH2. After extraction with ethyl acetate. the organic layer was washed with water and then brine, dried ($MgSO_4$) and the solvent evaporated. The solid was filtered off. washed with hexane and dried under vacuum to give 5-benzyloxy-2-fluoro-4-methylnitrobenzene (4.83g, 80%).

The starting material 2-fluoro-5-hydroxy-4-methylnitrobenzene. was obtained by adding 2M aqueous sodium hydroxide solution (13.1ml), dropwise. to a solution of 2-fluoro-5-methoxycarbonyloxy-4-methylnitrobenzene (6g, 26mmol), (prepared as described in European Patent Publication No. 307777), in methanol (70ml) cooled at 0°C. After stirring for 30 minutes, the mixture was concentrated by evaporation. After dilution with water. the solution was adjusted to pH2 and extracted with ethyl acetate. The organic layer was washed with water and then brine. dried ($MgSO_4$) and the solvent evaporated to give 2-fluoro-5-hydroxy-4-methylnitrobenzene as a yellow solid (4g. 90%).

Example 21

A solution of 4-chloro-6.7-dimethoxycinnoline hydrochloride (261mg, 1mmol), (prepared as described for the starting material in Example 1), and 2-fluoro-5-hydroxy-4-methylaniline (170mg, 1.2mmol), (prepared as described for the starting material in Example 11), in 2-pentanol (5ml) was heated at 120°C for 3 hours. After cooling, the solid was filtered off, washed with isopropanol and ether and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6,7-dimethoxycinnoline** as an hydrochloride salt (301mg, 82%).
m.p. 251-253°C

^{1}H NMR Spectrum: (DMSOd_6; CD_3COOD) 2.22(s, 3H); 4.05(s, 3H); 4.07(s, 3H); 6.97(d, 1H); 7.24(d, 1H); 7.39(s, 1H); 8.08(s, 1H); 8.22(d, 1H)

MS - ESI m/z: 330 $[MH]^+$

Elemental analysis:	Found	C 55.2	H 4.8	N 11.1
$C_{17}H_{16}N_3O_3F$ 0.1H_2O 1HCl	Requires	C 55.6	H 4.7	N 11.4%

Example 22

A solution of 4-chloro-6,7-dimethoxycinnoline hydrochloride (261mg, 1mmol), (prepared as described for the starting material in Example 1), and 4-chloro-2-fluoro-5-hydroxyaniline (193mg, 1.2mmol), (prepared as described in EP 061741), was treated in a manner similar to that described in Example 21 in order to produce **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6,7-dimethoxycinnoline** as an hydrochloride salt (315mg, 82%).
m.p. 255-256°C

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 4.05(s, 3H); 4.07(s, 3H); 7.19(d, 1H); 7.4(s, 1H); 7.65(d, 1H); 8.07(s, 1H); 8.34(d, 1H)

MS - ESI m/z: 350 $[MH]^+$

Elemental analysis:	Found	C 49.5	H 3.8	N 10.5
$C_{16}H_{13}N_3O_3ClF$ 1HCl	Requires	C 49.8	H 3.7	N 10.9%

Example 23

A solution of 4-chloro-6-methoxy-7-[2-(2-methoxyethoxy)ethoxy]cinnoline (130mg, 0.41mmol) and 2-fluoro-5-hydroxy-4-methylaniline (70mg, 0.5mmol), (prepared as described

for the starting material in Example 11), in 2-pentanol (3ml) containing 5M hydrogen chloride in isopropanol (2drops) was heated at reflux for 45 minutes. After cooling the solid was filtered off, washed with isopropanol followed by ether to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-[2-(2-methoxyethoxy)ethoxy]cinnoline** as an hydrochloride salt (159mg, 88%).

m.p. 250-256°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.21(s, 3H); 3.27(s, 3H); 3.51(dd, 2H); 3.66(dd, 2H); 3.90(t, 2H); 4.06(s, 3H); 4.37(t, 2H); 6.98(d, 1H); 7.25(d, 1H); 7.46(s, 1H); 8.18(d, 1H); 8.23(s, 1H); 9.94(s, 1H); 11.2(s, 1H)

MS - ESI m/z: 418 $[MH]^+$

Elemental analysis:	Found	C 55.2	H 5.7	N 8.8
$C_{21}H_{24}N_3O_5F$ 0.2H_2O 1HCl	Requires	C 55.1	H 5.6	N 9.2%

The starting compound 4-chloro-6-methoxy-7-[2-(2-methoxyethoxy)ethoxy]-cinnoline was obtained by adding triphenylphosphine (995mg, 3.8mmol), followed by diethyleneglycol (271µl, 2.2mmol) and diethyl azodicarboxylate (598µl, 3.8mmol), dropwise, to a suspension of 4-chloro-7-hydroxy-6-methoxycinnoline (0.4g, 1.9mmol), (prepared as described for the starting material in Example 10), in methylene chloride (12ml) under nitrogen and cooled to 10°C. After stirring for 1 hour, the solvent was evaporated and the residue purified by flash chromatography using methylene chloride/ethyl acetate (5/5 followed by 4/6) as eluent to give 4-chloro-6-methoxy-7-[2-(2-methoxyethoxy)ethoxy]cinnoline (366mg, 91%).

Example 24

A solution of 4,6-dichlorocinnoline (200mg, 1mmol) and 2-fluoro-5-hydroxy-4-methylaniline (169mg, 1.2mmol), (prepared as described for the starting material in Example 11), in 2-pentanol (4ml) containing 7M isopropanolic hydrogen chloride (2drops) was heated at reflux for 45 minutes. After cooling the solid was filtered off, washed with isopropanol followed by ether to give **6-chloro-4-(2-fluoro-5-hydroxy-4-methylanilino)cinnoline** hydrochloride (326mg, 95%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.20(s, 3H); 6.98(d, 1H); 7.26(d, 1H); 8.18(s, 2H);

8.35(s, 1H); 9.1(s, 1H); 9.98(br s, 1H)

MS - ESI: 304 $[MH]^+$

Elemental Analysis:	Found	C 53.4	H 4.0	N 11.9
$C_{15}H_{11}N_3OFCl\ 0.95HCl\ 0.1H_2O$	Requires	C 53.0	H 3.6	N 12.4%

The starting material was prepared as follows:

A solution of 4-hydroxy-6-chlorocinnnoline (1g, 5.5mmol), (J. Chem. Soc. 1961, 1828), in thionyl chloride (10ml) and DMF (0.1ml) was heated at reflux for 20 minutes. Toluene was added and the volatiles were removed by evaporation. The solid was partitioned between ethyl acetate and water and the aqueous layer adjusted to pH7 with sodium hydrogen carbonate. The organic layer was washed with water and brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/ether (95/5) to give 4,6-dichlorocinnoline (804mg, 73%).

m.p. 111-113°C

^1H NMR Spectrum: ($CDCl_3$) 7.85(dd, 1H); 8.18(d, 1H); 8.52(d, 1H); 9.36(s, 1H)

MS - EI: 199 $[M\cdot]^+$

Elemental Analysis:	Found	C 48.5	H 2.1	N 14.0
$C_8H_4N_2Cl_2$	Requires	C 48.3	H 2.0	N 14.1%

Example 25

A solution of 4-chloro-6-methoxy-7-(3-morpholinopropoxy)cinnoline hydrochloride (150mg, 0.36mmol), (prepared as described for the starting material in Example 12), 4-chloro-2-fluoroaniline (77mg, 0.53mmol) in 2-pentanol (4ml) and 5M isopropanolic hydrogen chloride (1ml) was heated at 120°C for 1 hour. The mixture was allowed to cool and isopropanol was added. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)cinnoline** hydrochloride (185mg, 98%).

^1H NMR Spectrum: ($DMSOd_6$) 2.3-2.4(m, 2H); 3.1-3.2(m, 2H); 3.3-3.4(m, 2H); 3.5-3.6(m, 2H); 3.8-3.95(m, 2H); 3.95-4.1(m, 2H); 4.08(s, 3H); 4.38(t, 2H); 7.55(dd, 1H); 7.57(s, 1H); 7.71(t, 1H); 7.80(dd, 1H); 8.31(d, 1H); 8.4(s, 1H)

MS - ESI: 447 $[MH]^+$

Elemental Analysis:	Found	C 50.0	H 5.2	N 10.2
$C_{22}H_{24}N_4O_2FCl$ 1.95HCl 0.5H_2O	Requires	C 50.1	H 5.1	N 10.6%

Example 26

A solution of 4-chloro-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline hydrochloride (130mg, 0.32mmol), (prepared as described for the starting material in Example 13), and 4-chloro-2-fluoroaniline (70mg, 0.48mmol), in 2-pentanol (4ml) and 5M isopropanolic hydrogen chloride (1ml) was heated at 120°C for 2.5 hours. The solid was filtered off, washed with isopropanol and then ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline** hydrochloride (110mg, 66%).

^{1}H NMR Spectrum: (DMSOd_6; CD_3COOD) 1.9-2.1(m, 4H); 2.25-2.35(m, 2H); 3.0-3.3(br m, 2H); 3.36(t, 2H); 3.4-3.7(m, 2H); 4.06(s, 3H); 4.38(t, 2H); 7.46(s, 1H); 7.55(d, 1H); 7.72(t, 1H); 7.75(dd, 1H); 8.21(s, 1H); 8.33(s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental Analysis:	Found	C 51.2	H 5.4	N 10.3
$C_{22}H_{24}N_4O_2FCl$ 1.9HCl 1.1H_2O	Requires	C 50.8	H 5.4	N 10.8%

Example 27

A solution of 4-chloro-7-(2-methoxyethoxy)cinnoline (156mg, 0.65mmol) and 2-fluoro-5-hydroxy-4-methylaniline (111mg, 0.78mmol), (prepared as described for the starting material in Example 11), in 2-pentanol (8ml) and 5M isopropanolic hydrogen chloride (1ml) was heated at 120°C for 2.5 hours. The solid was filtered off, washed with isopropanol and then ether and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethoxy)cinnoline** hydrochloride (207mg, 84%).

^{1}H NMR Spectrum: (DMSOd_6) 2.19(s, 3H); 3.4(s, 3H); 3.8(t, 2H); 4.4(t, 2H); 6.96(d, 1H); 7.25(d, 1H); 7.38(s, 1H); 7.61(d, 1H); 8.21(s, 1H); 8.71(d, 1H); 9.94(s, 1H)

MS - ESI: 344 $[MH]^+$

Elemental Analysis:	Found	C 57.1	H 5.4	N 11.3
$C_{18}H_{18}N_3O_3F$ 0.15H_2O 0.95HCl	Requires	C 56.8	H 5.1	N 11.0%

The starting material was prepared as follows:

Diethyl azodicarboxylate (349µl, 2.2mmol) was added dropwise to a suspension of 4-chloro-7-hydroxycinnnoline (200mg, 1.1mmol), triphenylphosphine (580mg, 2.2mmol) and 2-methoxyethanol (105µl, 1.3mmol) in methylene chloride (6ml) cooled at 10°C. The mixture was stirred for 30 minutes and further triphenylphosphine (145mg), 2-methoxyethanol (20µl) and diethyl azodicarboxylate (88µl) were added. The mixture was then allowed to warm to ambient temperature and stirred for 30 minutes. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate/petroleum ether (7/3) and followed by column chromatography eluting with cyclohexane/ethyl acetate 35/65 to give 4-chloro-7-(2-methoxyethoxy)cinnoline (158mg, 60%).

^{1}H NMR Spectrum: ($CDCl_3$) 3.5(s, 3H); 3.88(m, 2H); 4.36(m, 2H); 7.58(dd, 1H); 7.77(d, 1H); 8.08(d, 1H); 9.2(s, 1H)

Aluminium trichloride (2.6g, 19mmol) was added in portions to a suspension of 4-chloro-7-methoxycinnoline (0.9g, 3.8mmol), (J. Chem. Soc. 1955, 2100), in benzene (15ml) and the mixture was heated at reflux for 1 hour. The solvent was removed by evaporation and the residue was partitioned between ice/water and ethyl acetate. Aqueous saturated sodium chloride solution was added and the organic layer was separated. The organic layer was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with ether, collected, filtered and dried under vacuum to give 4-chloro-7-hydroxycinnoline (368mg, 53%).

^{1}H NMR Spectrum: ($DMSOd_6$) 7.6(dd, 1H); 7.66(d, 1H); 8.11(d, 1H); 9.35(s, 1H)

Example 28

The following illustrate representative pharmaceutical dosage forms containing the compound of formula I, or a pharmaceutically acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

(a) Tablet I

	mg/tablet
Compound X	100
Lactose Ph.Eur	182.75
Croscarmellose sodium	12.0
Maize starch paste (5% w/v paste)	2.25
Magnesium stearate	3.0

(b) Tablet II

	mg/tablet
Compound X	50
Lactose Ph.Eur	223.75
Croscarmellose sodium	6.0
Maize starch	15.0
Polyvinylpyrrolidone (5% w/v paste)	2.25
Magnesium stearate	3.0

(c) Tablet III

	mg/tablet
Compound X	1.0
Lactose Ph.Eur	93.25
Croscarmellose sodium	4.0
Maize starch paste (5% w/v paste)	0.75
Magnesium stearate	1.0

(d) Capsule

	mg/capsule
Compound X	10
Lactose Ph.Eur	488.5
Magnesium stearate	1.5

(e) Injection I

	(50 mg/ml)
Compound X	5.0% w/v
1N Sodium hydroxide solution	15.0% v/v

0.1N Hydrochloric acid
(to adjust pH to 7.6)
Polyethylene glycol 400 ..... 4.5% w/v
Water for injection to 100%

(f) Injection II (10 mg/ml)

Compound X ..... 1.0% w/v
Sodium phosphate BP ..... 3.6% w/v
0.1N Sodium hydroxide solution..... 15.0% v/v
Water for injection to 100%

(g) Injection III (1mg/ml.buffered to pH6)

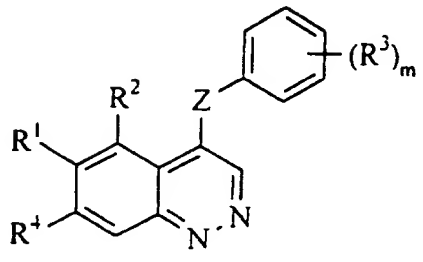
Compound X ..... 0.1% w/v
Sodium phosphate BP ..... 2.26% w/v
Citric acid ..... 0.38% w/v
Polyethylene glycol 400 ..... 3.5% w/v
Water for injection to 100%

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

CLAIMS

1. The use of a compound of the formula I:



(I)

[wherein:

Z represents -O-, -NH-, -S- or $-CH_2-$;

m is an integer from 1 to 5;

R^1 represents hydrogen, hydroxy, halogeno, nitro, cyano, trifluoromethyl, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio or NR^6R^7 (wherein R^6 and R^7, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, fluoro, chloro, methoxy, amino or nitro;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

R^4 represents hydrogen, hydroxy, halogeno, cyano, nitro, amino, trifluoromethyl, C_{1-3}alkyl or a group R^5-X^1 (wherein X^1 represents -O-, $-CH_2-$, -S-, -SO-, $-SO_2-$, $-NR^8CO-$, $-CONR^9-$, $-SO_2NR^{10}-$, $-NR^{11}SO_2-$ or $-NR^{12}-$ (wherein R^8, R^9, R^{10}, R^{11} and R^{12} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^5 is selected from one of the following fourteen groups:

1) C_{1-5}alkyl, C_{1-5}hydroxyalkyl, C_{1-5}fluoroalkyl, C_{1-5}aminoalkyl;

2) C_{1-5}alkylX^2COR^{13} (wherein X^2 represents -O- or NR^{14} (in which R^{14} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxyC_{2-3}alkyl) and R^{13} represents C_{1-3}alkyl, $NR^{15}R^{16}$ or OR^{17} (wherein R^{15}, R^{16} and R^{17} which may be the same or different are each hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl), with the proviso that when X^2 is -O-, R^{13} is not OR^{17});

3) C_{1-5}alkylX^3R^{18} (wherein X^3 represents -O-, -S-, -SO-, $-SO_2-$, -OCO-, $-NR^{19}CO-$, $-CONR^{20}-$, $-SO_2NR^{21}-$, $-NR^{22}SO_2-$ or $-NR^{23}-$ (wherein R^{19}, R^{20}, R^{21}, R^{22} and R^{23} each

independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{18} represents hydrogen, C_{1-3}alkyl, cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-4}alkoxy and which cyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

4) C_{1-5}alkylX^4C_{1-5}alkylX^5R^{24} (wherein X^4 and X^5 which may be the same or different are each -O-, -S-, -SO-, $-SO_2-$, $-NR^{25}CO-$, $-CONR^{26}-$, $-SO_2NR^{27}-$, $-NR^{28}SO_2-$ or $-NR^{29}-$ (wherein R^{25}, R^{26}, R^{27}, R^{28} and R^{29} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{24} represents hydrogen or C_{1-3}alkyl);

5) C_{1-5}alkylR^{30} (wherein R^{30} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

6) C_{2-5}alkenylR^{30} (wherein R^{30} is as defined herein);

7) C_{2-5}alkynylR^{30} (wherein R^{30} is as defined herein);

8) $(CH_2)_nR^{31}$ (wherein n is an integer from 0 to 5 and R^{31} is a phenyl group, a pyridone group or a 5 or 6 membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which phenyl, pyridone or aromatic heterocyclic group may carry up to 5 substituents selected from hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}hydroxyalkoxy, carboxy, cyano, $CONR^{32}R^{33}$ and $NR^{34}COR^{35}$ (wherein R^{32}, R^{33}, R^{34} and R^{35}, which may be the same or different, each represents hydrogen, C_{1-4}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

9) C_{2-5}alkenylR^{31} (wherein R^{31} is as defined herein);

10) C_{2-5}alkynylR^{31} (wherein R^{31} is as defined herein);

11) C_{1-5}alkylX^6R^{31} (wherein X^6 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{36}CO-$, $-CONR^{37}-$, $-SO_2NR^{38}-$, $-NR^{39}SO_2-$ or $-NR^{40}-$ (wherein R^{36}, R^{37}, R^{38}, R^{39} and R^{40} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{31} is as defined herein);

12) C_{2-5}alkenylX^7R^{31} (wherein X^7 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{41}CO-$, $-CONR^{42}-$, $-SO_2NR^{43}-$, $-NR^{44}SO_2-$ or $-NR^{45}-$ (wherein R^{41}, R^{42}, R^{43}, R^{44} and R^{45} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{31} is as defined herein);

13) C_{2-5}alkynylX^8R^{31} (wherein X^8 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{46}CO-$, $-CONR^{47}-$, $-SO_2NR^{48}-$, $-NR^{49}SO_2-$ or $-NR^{50}-$ (wherein R^{46}, R^{47}, R^{48}, R^{49} and R^{50} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{31} is as defined herein);

14) C_{1-3}alkylX^9C_{1-3}alkylR^{31} (wherein X^9 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{51}CO-$, $-CONR^{52}-$, $-SO_2NR^{53}-$, $-NR^{54}SO_2-$ or $-NR^{55}-$ (wherein R^{51}, R^{52}, R^{53}, R^{54} and R^{55} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{31} is as defined herein))];

and salts thereof, in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

2. A compound of the formula I as defined in claim 1 with the proviso that where m is 1, R^3 is meta-hydroxy and with the further proviso that the compound of formula I is not 4-(2,6-dimethylphenoxy)-6,7-dimethoxycinnoline; and salts thereof, for use as a medicament.

3. A compound of the formula I as defined in claim 1 with the proviso that where m is 1, R^3 is meta-hydroxy and with the further proviso that the phenyl group bearing $(R^3)_m$ is not 3,4-dimethylphenyl and that when the phenyl group bearing $(R^3)_m$ is 2,5-dichlorophenyl, 3,5-dichlorophenyl, 2,3-dichlorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl, 2,6-dichlorophenyl, 2,6-dimethylphenyl, 2-bromo-4-chlorophenyl, 4-bromo-2-chlorophenyl, 2-bromo-4-methylphenyl, 2-chloro-4-methylphenyl, 2-chloro-4-hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 3,5-dichloro-4-hydroxyphenyl, 2,5-dichloro-4-hydroxyphenyl or 5-chloro-2-methylphenyl, Z is -NH-; and salts thereof.

4. A cinnoline derivative as claimed in claim 3 wherein R^2 is hydrogen.

5. A cinnoline derivative as claimed in claim 3 or claim 4 wherein Z is -NH-.

6. A cinnoline derivative as claimed in any one of claims 3 to 5 wherein R^1 is methoxy.

7. A cinnoline derivative as claimed in any one of claims 3 to 6 wherein the phenyl group bearing $(R^3)_m$ is 2-fluoro-5-hydroxy-4-methylphenyl, 4-chloro-2-fluoro-5-hydroxyphenyl, 4-chloro-3-hydroxyphenyl, 4-bromo-3-hydroxyphenyl, 3-hydroxy-4-methylphenyl,or 4-bromo-2-fluoro-5-hydroxyphenyl group.

8. A cinnoline derivative as claimed in any one of claims 3 to 7 wherein R^4 is hydroxy, halogeno, nitro, trifluoromethyl, C_{1-3}alkyl, cyano, amino or a group R^5-X^1 (wherein X^1 is as defined in claim 1 and R^5 is selected from one of the following fourteen groups:

1) C_{1-4}alkyl, C_{2-4}hydroxyalkyl, C_{1-4}fluoroalkyl, C_{2-4}aminoalkyl;

2) C_{2-3}alkylX^2COR^{13} (wherein X^2 is as defined in claim 1 and R^{13} represents C_{1-3}alkyl, $NR^{15}R^{16}$ or OR^{17} (wherein R^{15}, R^{16} and R^{17} which may be the same or different are each C_{1-2}alkyl or C_{1-2}alkoxyethyl) with the proviso that when X^2 is -O-, R^{13} is not OR^{17});

3) C_{2-4}alkylX^3R^{18} (wherein X^3 is as defined in claim 1 and R^{18} represents C_{1-3}alkyl, cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^3 through a carbon atom and which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-2}alkoxy and which cyclopentyl, cyclohexyl, pyrrolidinyl or piperidinyl group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

4) C_{2-3}alkylX^4C_{2-3}alkylX^5R^{24} (wherein X^4 and X^5 are as defined in claim 1 and R^{24} represents hydrogen or C_{1-3}alkyl);

5) C_{1-4}alkylR^{59} (wherein R^{59} is a group selected from pyrrolidinyl, piperazinyl, piperidinyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, which group is linked to C_{1-4}alkyl through a carbon atom and which group may carry one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy) or C_{2-4}alkylR^{60} (wherein R^{60} is a group selected from morpholino, thiomorpholino, pyrrolidin-1-yl, piperazin-1-yl and piperidino which group may carry one or two

substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

6) C_{3-4}alkenylR^{61} (wherein R^{61} represents R^{59} or R^{60} as defined herein);

7) C_{3-4}alkynylR^{61} (wherein R^{61} represents R^{59} or R^{60} as defined herein);

8) $(CH_2)_nR^{31}$ (wherein n is an integer from 1 to 3 and R^{31} is a pyridone group or a 5 or 6 membered aromatic heterocyclic group with 1 to 2 heteroatoms selected from O, N and S, of which one is N which pyridone or aromatic heterocyclic group may be substituted with one substituent selected from halogeno, C_{1-2}alkyl, C_{1-2}alkoxy, C_{1-2}hydroxyalkyl, C_{1-2}hydroxyalkoxy, carboxy, cyano, $CONR^{32}R^{33}$ and $NR^{34}COR^{35}$ (wherein R^{32}, R^{33}, R^{34} and R^{35}, which may be the same or different, each represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl));

9) 1-R^{31}but-2-en-4-yl (wherein R^{31} is as defined herein);

10) 1-R^{31}but-2-yn-4-yl (wherein R^{31} is as defined herein);

11) C_{1-5}alkylX^6R^{31} (wherein X^6 is as defined in claim 1 and R^{31} is as defined herein);

12) 1-($R^{31}X^7$)but-2-en-4-yl (wherein X^7 is as defined in claim 1 and R^{31} is as defined herein);

13) 1-($R^{31}X^8$)but-2-yn-4-yl (wherein X^8 is as defined in claim 1 and R^{31} is as defined herein);

14) C_{2-3}alkylX^9C_{1-2}alkylR^{31} (wherein X^9 is as defined in claim 1 and R^{31} is as defined herein)).

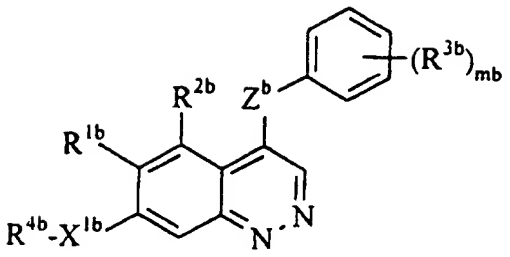
9. A cinnoline derivative as claimed in any one of claims 3 to 8 wherein R^4 is hydroxy, C_{1-3}alkyl, amino, or a group R^5-X^1 (wherein X^1 is as defined in claim 1 and R^5 is methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-

hydroxyethylamino)propyl, 2-methylthiazol-4-ylmethyl, 2-acetamidothiazol-4-ylmethyl, 1-methylimidazol-2-ylmethyl, 2-(imidazol-1-yl)ethyl, 2-(1,2,3-triazol-1-yl)ethyl, 2-(1,2,3-triazol-2-yl)ethyl, 2-(1,2,4-triazol-1-yl)ethyl, 2-(1,2,4-triazol-4-yl)ethyl, 4-pyridylmethyl, 2-(4-pyridyl)ethyl, 3-(4-pyridyl)propyl, 2-(4-pyridyloxy)ethyl, 2-(4-pyridylamino)ethyl, 2-(4-oxo-1,4-dihydro-1-pyridyl)ethyl or 2-thiomorpholinoethyl, 3-thiomorpholinopropyl, 2-(2-methoxyethoxy)ethyl, 2-(4-methylpiperazin-1-yl)ethyl or 3-(4-methylpiperazin-1-yl)propyl).

10. A cinnoline derivative as claimed in any one of claims 3 to 9 wherein R^4 is methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, 2-(1,3-dioxolan-2-yl)methyl, 3-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-methylthiazol-4-ylmethyl, 1-methylimidazol-2-ylmethyl, 4-pyridylmethyl, 2-(4-pyridyl)ethyl or 3-(4-pyridyl)propyl.

11. A cinnoline derivative as claimed in any one of claims 3 to 7 wherein R^4 is a group R^5-X^1 in which R^5 is as defined in claim 1 and X^1 represents -O-, -S-, -NR^8CO- (wherein R^8 represents hydrogen or methyl) or NH.

12. A compound of the formula Ib:



(Ib)

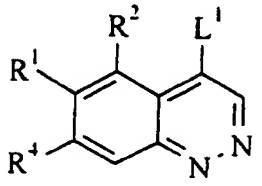
wherein R^{1b} is hydrogen, C_{1-3}alkoxy, or halogeno; R^{2b} is hydrogen; X^{1b} is -O-; R^{4b} is C_{1-3}alkyl, 2-(C_{1-3}alkoxy)ethyl, benzyl, 4-pyridyl(C_{1-3}alkyl), morpholino(C_{1-3}alkyl), pyrrolidino(C_{1-3}alkyl), 2-methylthiazol-4-yl(C_{1-3}alkyl), 1-methylimidazol-2-yl(C_{1-3}alkyl) and 2-((C_{1-3}alkoxy)(C_{1-3}alkoxy))ethyl; Z^b is -NH- or -O-; mb is 2 or 3; and the phenyl group bearing $(R^{3b})_{mb}$ is selected from: 3-hydroxy-4-methylphenyl, 4-chloro-2-fluorophenyl, 4-bromo-2-fluorophenyl, 4-chloro-2-fluoro-5-hydroxyphenyl, 5-acetoxy-4-chloro-2-fluorophenyl, 2-fluoro-5-hydroxy-4-methylphenyl and 4-bromo-2-fluoro-5-hydroxyphenyl; and salts thereof.

13. A cinnoline derivative as claimed in claim 1 selected from:
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline,
4-(4-bromo-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)cinnoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)cinnoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-[(2-methylthiazol-4-yl)methoxy]cinnoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-pyrrolidinopropoxy)cinnoline
and salts thereof.

14. A cinnoline derivative as claimed in any one of the claims 3 to 13 in the form of a pharmaceutically acceptable salt.

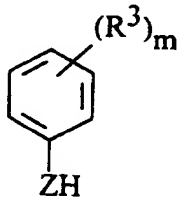
15. A process for the preparation of a cinnoline derivative of formula I or salt thereof (as defined in claim 3) which comprises:

(a) the reaction of a compound of the formula III:



(III)

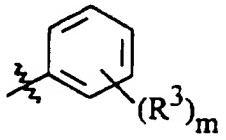
(wherein R^1, R^2 and R^4 are as defined in claim 1 and L^1 is a displaceable moiety), with a compound of the formula IV:



(IV)

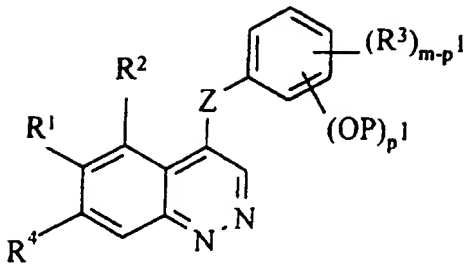
(wherein Z is as defined in claim 1 and R^3 and m are as defined in claim 3) whereby to obtain compounds of the formula I and salts thereof;

(b) for the preparation of compounds of formula I and salts thereof in which the group of formula IIa:



(IIa)

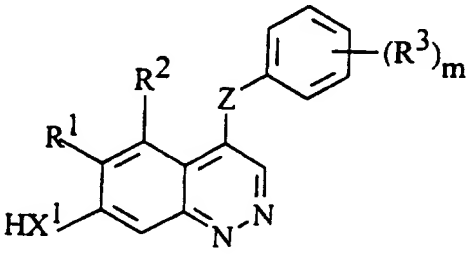
(wherein R^3 and m are as defined in claim 3) represents a phenyl group carrying one or more hydroxy groups, the deprotection of a compound of formula V:



(V)

wherein R^1, R^2, R^4 and Z are as defined in claim 1. R^3 and m are as defined in claim 3, P represents a phenolic hydroxy protecting group and p^1 is an integer from 1 to 5 equal to the number of protected hydroxy groups and such that $m-p^1$ is equal to the number of R^3 substituents which are not protected hydroxy;

(c) for the preparation of those compounds of formula I and salts thereof wherein the substituent R^4 represents R^5-X^1 in which R^5 is as defined in claim 1 and X^1 is -O-, -S- or -NR^{12}- (wherein R^{12} is as defined in claim 1), the reaction of a compound of the formula VI:



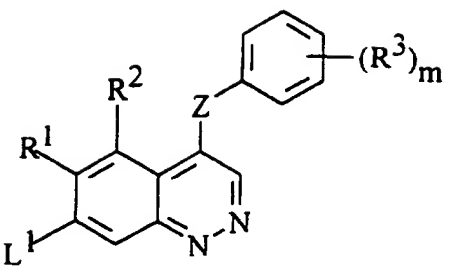
(VI)

wherein X^1 is as defined herein, R^1, R^2, and Z are as defined in claim 1 and R^3 and m are as defined in claim 3 with a compound of formula VII:

$$R^5\text{-}L^1 \qquad \text{(VII)}$$

wherein R^5 is as defined in claim 1 and L^1 is as defined herein;

(d) for the preparation of those compounds of the formula I and salts thereof wherein the substituent R^4 represents R^5-X^1 the reaction of a compound of the formula VIII:



(VIII)

with a compound of the formula IX:

$$R^5\text{-}X^1\text{-}H \qquad \text{(IX)}$$

(wherein R^1, R^2, R^5, Z and X^1 are all as defined in claim 1, R^3 and m are as defined in claim 3 and L^1 is as defined herein;

(e) for the preparation of those compounds of the formula I and salts thereof wherein R^4 represents R^5-X^1 in which X^1 is as defined in claim 1 and R^5 is $C_{1-5}alkylR^{64}$, (wherein R^{64} is selected from one of the following four groups:

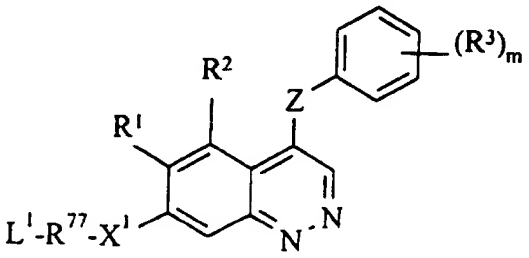
1) $X^{10}C_{1-3}alkyl$ (wherein X^{10} represents -O-, -S-, $-SO_2-$, $NR^{65}CO$, $NR^{66}SO_2$ or NR^{67} (wherein R^{65}, R^{66} and R^{67} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$));

2) $NR^{68}R^{69}$ (wherein R^{68} and R^{69} which may be the same or different are each hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$);

3) $X^{11}C_{1-5}alkylX^5R^{24}$ (wherein X^{11} is -O-, -S-, $-SO_2-$, $NR^{70}CO$, $NR^{71}SO_2$ or NR^{72} (wherein R^{70}, R^{71} and R^{72} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and X^5 and R^{24} are as defined in claim 1); and

4) an aromatic heterocyclic group selected from pyrrolyl, imidazolyl, pyrazolyl and triazolyl (which aromatic heterocyclic group is linked to the $C_{1-5}alkyl$ moiety via a nitrogen atom of the heterocyclic ring and which aromatic heterocyclic group may carry up to 4 substituents selected from halogeno, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}hydroxyalkyl$, $C_{1-4}hydroxyalkoxy$, carboxy, cyano, $CONR^{73}R^{74}$ and $NR^{75}COR^{76}$ (wherein R^{73}, R^{74}, R^{75} and R^{76}, which may be the same or different, each represents hydrogen or $C_{1-4}alkyl$)), or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is nitrogen and the other one may be selected independently from O, S and N, (which heterocyclic group is linked to the $C_{1-5}alkyl$ moiety via a nitrogen atom of the heterocyclic

group and which heterocyclic group may bear one or two substituents selected from halogeno, C_{1-4}alkyl. C_{1-4}hydroxyalkyl and C_{1-4}alkoxy)), by reacting a compound of the formula X:



(X)

(wherein X^1, R^1, R^2 and Z are as defined in claim 1, R^3 and m are as defined in claim 3. L^1 is as defined herein and R^{77} is C_{1-5}alkyl) with a compound of the formula XI:

$$R^{64}\text{-}H \qquad \text{(XI)}$$

(wherein R^{64} is as defined herein);

and when a salt of a cinnoline derivative of formula I is required, reaction of the compound obtained with an acid or base whereby to obtain the desired salt.

16. A pharmaceutical composition which comprises as active ingredient a cinnoline derivative of formula I or a pharmaceutically acceptable salt thereof as claimed in any one of claims 3 to 14 in association with a pharmaceutically acceptable excipient or carrier.

17. A method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as claimed in claim 1.

INTERNATIONAL SEARCH REPORT

Interr ial Application No
PCT/GB 97/00650

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D237/28 C07D417/12 C07D401/12 C07D403/12 A61K31/495

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 326 330 A (LILLY) 2 August 1989 cited in the application see page 20 - page 26; claims ---	1,2,4,6
A	CHEMICAL ABSTRACTS, vol. 73, no. 15, 1970 Columbus, Ohio, US; abstract no. 77174z, S.M. YARNAL ET AL.: "CINNOLINES.2" page 366; column 2; XP002032600 see abstract & ARCH. PHARM., vol. 303, no. 7, 1970, WEINHEIM, pages 560-562, cited in the application --- -/--	1,2,6 1,2,6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
9 June 1997	13.06.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	**Authorized officer** Francois, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 97/00650

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 79, no. 9, 1973 Columbus, Ohio, US; abstract no. 53246x, S.M. YARNAL ET AL.: "CINNOLINE CHEMISTRY.III." page 360; column 2; XP002032601 see abstract & INDIAN J. CHEM., vol. 11, no. 3, 1973, INDIA, pages 211-213, cited in the application	1,2,6
A	CHEMICAL ABSTRACTS, vol. 82, no. 28, 1975 Columbus, Ohio, US; abstract no. 43302k, S.M. YARNAL ET AL.: "CINNOLINE CHEMISTRY.IV." page 431; column 1; XP002032602 see abstract & J. KARNATAK UNIV., vol. 18, 1973, KHARWAR, pages 25-30, cited in the application	1,2,6
A	CHEMICAL ABSTRACTS, vol. 104, no. 25, 1986 Columbus, Ohio, US; abstract no. 224864p, S.M. YARNAL ET AL.: "STUDIES IN CINNOLINE CHEMISTRY.V." page 612; column 1; XP002032603 cited in the application see abstract & J. KARNATAK UNIV. SCI., vol. 29, 1984, DHARWAD, pages 82-86, cited in the application	1,2,4,6
A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 11, 1968, WASHINGTON US, page 1270 XP002032599 S.M. YARNAL ET AL.: "STUDIES IN CINNOLINE CHEMISTRY.I. THE SYNTHESIS OF SUBSTITUTED PHENYL CINNOLYL SULFIDES." cited in the application see page 1270	1,2,6

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter nal Application No
PCT/GB 97/00650

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 326330 A	02-08-89	AU 2872889 A	03-08-89
		CN 1034925 A,B	23-08-89
		EG 18859 A	29-09-94
		FI 94523 B	15-06-95
		HU 208611 B	28-12-93
		JP 1246263 A	02-10-89
		JP 2559485 B	04-12-96
		US 5145843 A	08-09-92
		US 5240940 A	31-08-93

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/40

A1

(11) International Publication Number: WO 97/40830

(43) International Publication Date: 6 November 1997 (06.11.97)

(21) International Application Number: PCT/US97/07752

(22) International Filing Date: 1 May 1997 (01.05.97)

(30) Priority Data:

60/016,658	1 May 1996 (01.05.96)	US
Not furnished	30 April 1997 (30.04.97)	US

(71) Applicant *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: JIROUSEK, Michael, R. [US/US]; 10342 Fawn Ridge Lane, Indianapolis, IN 46236 (US). VIGNATI, Louis [US/US]; 5617 North Meridian, Indianapolis, IN 46208 (US). WAYS, Douglas, Kirk [US/US]; 4565 North Park Avenue, Indianapolis, IN 46205 (US).

(74) Agents: SKERPON, Joseph, M. et al.; Banner & Witcoff, Ltd., Suite 1100, 1001 G Street, N.W., Washington, DC 20001 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: THERAPEUTIC TREATMENT FOR VEGF RELATED DISEASES

(57) Abstract

A method for inhibiting VEGF stimulated endothelial cell growth, such as associated with neoplasia, and VEGF stimulated capillary permeability, such as associated with pulmonary edema are disclosed, particularly using the β-isozyme selective PKC inhibitor, (S)-3,4-[N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione hydrochloride salt.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

THERAPEUTIC TREATMENT FOR VEGF RELATED DISEASES

This application claims the priority benefit of the U.S. Provisional application Serial No. 60/016,658 filed May 1, 1996.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is broadly directed to a method for inhibiting endothelial cell growth and capillary permeability associated with vascular endothelial growth factor (VEGF), *e.g.*, the increased cell growth and permeability induced by (VEGF) using an inhibitor of the β isozyme of Protein Kinase C (PKC). This VEGF induced condition is closely associated with neoplasia in mammals and other disorders including pulmonary edema.

The present invention is particularly directed to the use of an inhibitor of the β isozyme of Protein Kinase C (PKC) for treating neoplastic diseases including capillary hemangioblastomia, breast cancer, Kaposi's sarcoma, glioblastoma, angiomatous disorders, colorectal cancer, medulloblastoma, gastric carcinoma, adenocarcinomas of the gastrointerestinal tract, malignant melanoma, ovarian cancer, non small cell lung cancer, prostate cancer, malignant effusions, preitumoral edema, *e.g.*, intracerebral edema and cysts associated with brain tumor, bladder carcinoma, von Hippel Lindau Syndrome, renal cell carcinoma, skin cancer, thyroid malignancies,

cervical cancer, hepatocellular carcinoma, rhabdomyosarcoma, and leiomysarcoma and certain other VEGF related disorders as described herein.

2. Description of Related Art

VPF/VEGF is a glycosylated, multifunctional cytokine. Over-expression of VPF/VEGF is associated with neoplasia, and several other disease conditions.

VPF/VEGF induces endothelial cell proliferation, excessive permeability via activation of vesicular-vacuolar organelle mediated transport, migration and actin reorganization with shape changes and ruffling. It alters endothelial cell gene expression, inducing increased production of tissue factor and several proteases, including interstitial collagenase and both the urokinase-like and tissue plasminogen activators. The majority of these same genes are induced by phorbol myristate acetate (PMA) stimulated activation of PKC.

VPF/VEGF is abundantly expressed and secreted by most human and animal tumors examined thus far. VPF/VEGF may directly affect tumor cells, *e.g.*, tumor cells of glioblastoma, as well as play an important role in the induction of tumor angiogenesis (Claffey, *et al.*, Cancer Research 56, 172-181 (1996) and the references cited therein).

The angiogenic potential of VEGF likely is enhanced by the synergistic activity of fibroblast growth factor liberated by cellular disruption or death. (Pepper, et al., *Biochem Biophys Res. Commun.*, 189:824-831 (1992); Muthukrishnan, et al., *J. Cell Physiol.*, 148:1-16 (1991)).

Tumor growth and metastasis are clos ly related to enhanced VEGF expression. A chemical signal from tumor cells can shift resting endothelial cells into

a phase of rapid growth. Of the twelve known angiogenic proteins, those most commonly found in tumors appear to be basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF) (Folkman, J. *New England J. of Medicine.*, Vol 999 (26): 1757-1763(1995) and the references cited therein).

The realization that tumor growth requires new blood vessels and the identification of chemical factors that mediate neovascularization or angiogenesis have broadened the understanding of pathologic processes and opened new avenues to the treatment of these diseases. Nine different inhibitors of angiogenesis are currently being studied in phase 1 or 2 clinical trials as treatment for a wide spectrum of solid tumors, including breast, colon, lung, and prostate cancer as well as Kaposi's sarcoma. (Folkman, J. Tumor angiogenesis In Mendelsohn J. Howley PM, Israel MA. Liotta LA. eds. The Molecular Basis of Cancer, Philadelphia: W.B. Saunders. 1995:206-232) One of these drugs, TNP-170, a synthetic analogue of fumagillin (Denekamp J. *Br J Radiol* 66:181-196, 1993) has been approved by the FDA for phase 1 testing in many patients with solid tumors. Other inhibitors of angiogenesis currently in clinical trials in patients with advanced cancer include platelet factor 4; carboxyaminotriazole; BB-94 and BB-2516; metalloproteinase inhibitors; the sulfated polysaccharide tecogalan (DS-152); thalidomide; interleukin-12; and linomide. (Flier et al., *The New England Journal of Medicine*, vol 333 pp1757-1763, 1995 and the references cited therein)

PKC inhibitors also have been proposed for cancer therapy, see U.S. 5,552,396. However, the effectiveness of the inhibitors of the β isozyme of PKC

against particular neoplastic diseases was not known. Given the role VEGF plays in certain neoplastic and other diseases, there is a need in the art to identify additional drugs that are specifically targeted at the function of VEGF.

SUMMARY OF INVENTION

It is an object of the invention to provide a method for treating neoplasia.

It is yet another object of the invention to provide a method for treating rheumatoid arthritis.

It is still yet another object of the invention to provide a method for treating keloid.

It is still another object of the invention to provide a method for treating pulmonary edema associated conditions, such as Adult Respiratory Distress Syndrome (ARDS).

It is still another object of the invention to provide a method for treating carpal tunnel syndrome.

These and other objects of the invention are provided by one or more of the embodiments described below.

In one embodiment of the invention there is provided a method for treating neoplasia which comprises administering to said mammal a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

In yet another embodiment of the invention there is provided a method for treating rheumatoid arthritis which comprises administering to said mammal a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

In another embodiment of the invention there is provided a method for treating keloid which comprises administering to said mammal a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

In yet another embodiment of the invention there is provided a method for treating pulmonary edema which comprises administering to said mammal a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

The present invention provides the art with the identity of compounds which are prophylactic and effective in treating neoplasia, and other disorders associated with vascular endothelial growth factor (VEGF).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the inhibitory effect of the PKC inhibitor, (S)-3,4-[N, N'-1,1'-((2"-ethoxy)-3'"(O)-4'"-(N,N-dimethylamino)-butane)-bis-(3,3'-indoly1)]-1(H)-pyrrole-2,5-dione on recombinant human VEGF stimulated endothelial cell growth.

Figure 2 further illustrates the inhibitory effect of the PKC inhibitor, (S)-3,4-[N, N'-1,1'-((2"-ethoxy)-3'"(O)-4'"-(N,N-dimethylamino)-butane)-bis-(3,3'-indoly1)]-1(H)-pyrrole-2,5-dione on recombinant human VEGF stimulated endothelial cell growth.

Figure 3 shows the impact of the PKC inhibitor on the activity of endogenous VEGF expressed upon culturing retinal pericytes under hypoxic conditions.

Figure 4 further illustrate the inhibitory effect of the PKC inhibitor on recombinant human VEGF stimulated endothelial cell growth.

DETAILED DESCRIPTION OF THE INVENTION

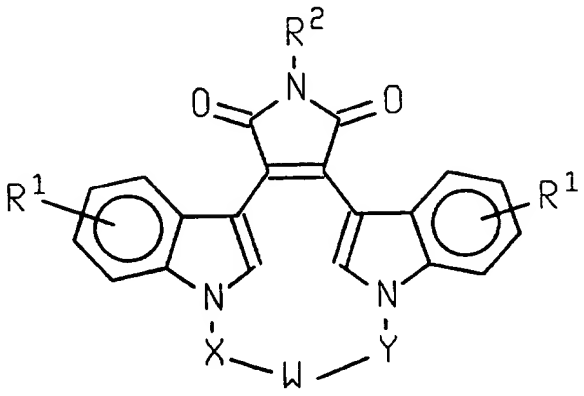
It is a discovery of the present invention that the therapeutic use of a particular class of protein kinase C inhibitors, *i.e.*, inhibitors of the β isozyme of protein kinase C, and especially β isozyme selective inhibitors of PKC, counteracts the effects of VEGF. In particular, it is a discovery of the present invention that use of this particular class of protein kinase C inhibitors counteracts endothelial cell growth and capillary permeability, especially the endothelial cell growth and the capillary permeability stimulated by the growth factor VEGF. Consequently, such compounds can be used therapeutically to treat disorders associated with VEGF, such as neoplasia, and other disease conditions that are associated with VEGF.

The method of this invention preferably utilizes those protein kinase C inhibitors that effectively inhibit the β isozyme. One suitable group of compounds are generally described in the prior art as bis-indolylmaleimides or macrocyclic bis-indolylmaleimides. Bis-indolylmaleimides well recognized in the prior art include those compounds described in U.S. Patents 5621098, 5552396, 5545636, 5481003, 5491242, and 5057614, all incorporated by reference herein. Macrocyclic bis-indolylmaleimides are particularly represented by the compounds of formula I. These compounds, and methods for their preparation, have been disclosed in U.S. Patent 5,552,396, which is incorporated herein by reference. These compounds are administered in a therapeutically effective amount to a mammal to inhibit endothelial cell growth or capillary permeability associated with VEGF, to inhibit VEGF effects associated with neoplasia, and other disease conditions, e.g., rheumatoid arthritis, keloid, carpal tunnel syndrome and pulmonary edema. These compounds can also be

administered to patients at risk of the disease conditions mentioned above as prophylactics.

One preferred class of compounds for use in the method of the invention has the formula:

(I)



wherein:

W is -O-, -S-, -SO-, -SO_2-, -CO-, C_2-C_6 alkylene, substituted alkylene, C_2-C_6 alkenylene, -aryl-, -aryl$(CH_2)_mO$-, -heterocycle-, -heterocycle-$(CH_2)_mO$-, -fused bicyclic-, -fused bicyclic-$(CH_2)_mO$-, -NR^3-, -NOR^3-, -CONH-, or -NHCO-;

X and Y are independently C_1-C_4 alkylene, substituted alkylene, or together X, Y, and W combine to form -$(CH_2)_n$-AA-;

R^1s are hydrogen or up to four optional substituents independently selected from halo, C_1-C_4 alkyl, hydroxy, C_1-C_4 alkoxy, haloalkyl, nitro, NR^4R^5, or -NHCO(C_1-C_4 alkyl);

R^2 is hydrogen, CH_3CO-, NH_2, or hydroxy;

R^3 is hydrogen, $(CH_2)_m$aryl, C_1-C_4 alkyl, -COO(C_1-C_4 alkyl), -$CONR^4R^5$, -$(C=NH)NH_2$, -SO(C_1-C_4 alkyl), -SO_2 (NR^4R^5), or -SO_2 (C_1-C_4 alkyl);

R^4 and R^5 are independently hydrogen, C_1-C_4 alkyl, phenyl, benzyl, or combine to the nitrogen to which they are bonded to form a saturated or unsaturated 5 or 6 member ring;

AA is an amino acid residue;

m is independently 0, 1, 2, or 3; and

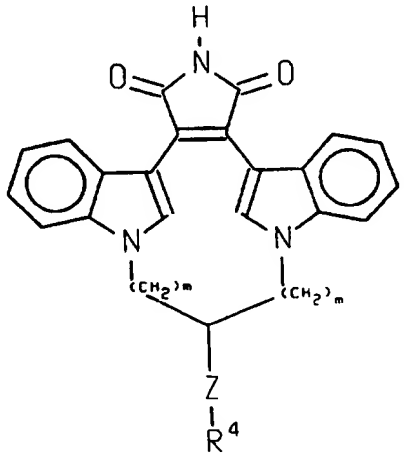
n is independently 2, 3, 4, or 5

or a pharmaceutically acceptable salt, prodrug or ester thereof.

A more preferred class of compounds for use in this invention is represented by formula I wherein the moieties -X-W-Y- contain 4 to 8 atoms, which may be substituted or unsubstituted. Most preferably, the moieties -X-W-Y- contain 6 atoms.

Other preferred compounds for use in the method of this invention are those compounds of formula I wherein R^1 and R^2 are hydrogen; and W is a substituted alkylene, -O-, S-, -CONH-, -NHCO- or -NR^3-. Particularly preferred compounds for use in the invention are compounds of the formula Ia:

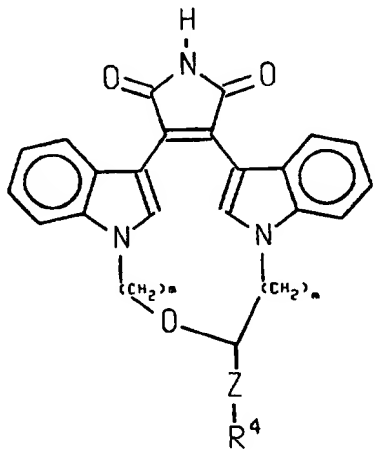
(Ia)



wherein Z is $-(CH_2)_p-$ or $-(CH_2)_p-O-(CH_2)_p-$; R^4 is hydroxy, -SH, C_1-C_4 alkyl, $(CH_2)_m$aryl, -NH(aryl), $-N(CH_3)(CF_3)$, $-NH(CF_3)$, or $-NR^5R^6$; R^5 is hydrogen or C_1-C_4 alky; R^6 is hydrogen, C_1-C_4 alkyl or benzyl; p is 0, 1, or 2; and m is independently 2 or 3 or a pharmaceutically acceptable salt, prodrug or ester thereof. Most preferred compounds of the formula Ia are those wherein Z is CH_2; and R^4 is $-NH_2$, $-NH(CF_3)$, or $-N(CH_3)_2$.

Other preferred compounds for use in the method of the present invention are compounds wherein W in formula I is -O-, Y is a substituted alkylene, and X is an alkylene. These preferred compounds are represented by formula Ib:

(Ib)



wherein Z is $-(CH_2)_p-$; R^4 is $-NR^5R^6$, $-NH(CF_3)$, or $-N(CH_3)(CF_3)$; R^5 and R^6 are independently H or C_1-C_4 alkyl; p is 0, 1, or 2; and m is independently 2 or 3 or a pharmaceutically acceptable salt, prodrug or ester thereof. Most preferred compounds of formula Ib are those wherein p is 1; and R^5 and R^6 are methyl.

Because they contain a basic moiety, the compounds of formulae I, Ia, and Ib can also exist as pharmaceutically acceptable acid addition salts. Acids commonly employed to form such salts include inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, methanesulfonic, oxalic, para-bromophenylsulfonic, carbonic, succinic, citric, benzoic, acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, mono-hydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, 2-butyne-1,4-dioate, 3-hexyne-2, 5-dioate, benzoate, chlorobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, hippurate, β-hydroxybutyrate, glycolate, maleate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like. Particularly the hydrochloric and mesylate salts are used.

In addition to pharmaceutically-acceptable salts, other salts also can exist. They may serve as intermediates in the purification of the compounds, in the preparation of other salts, or in the identification and characterization of the compounds or intermediates.

The pharmaceutically acceptable salts of compounds of formulae I, Ia, and Ib can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, ethyl acetate and the like. Mixtures of such solvates can also be

prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

It is recognized that various stereoisomeric forms of the compounds of formulae I, Ia, and Ib may exist; for example, W may contain a chiral carbon atom in the substituted alkylene moiety. The compounds are normally prepared as racemates and can conveniently be used as such. Alternatively, both individual enantiomers can be isolated or synthesized by conventional techniques if so desired. Such racemates and individual enantiomers and mixtures thereof form part of the compounds used in the methods of the present invention.

The compounds utilized in this invention also encompass the pharmaceutically acceptable prodrugs of the compounds of formulae I, Ia, and Ib. A prodrug is a drug which has been chemically modified and may be biologically inactive at its site of action, but which may be degraded or modified by one or more enzymatic or other *in vivo* processes to the parent bioactive form. This prodrug likely may have a different pharmacokinetic profile than the parent, enabling easier absorption across the mucosal epithelium, better salt formation or solubility, and/or improved systemic stability (an increase in plasma half-life, for example). Typically, such chemical modifications include the following:

1) ester or amide derivatives which may be cleaved by esterases or lipases;

2) peptides which may be recognized by specific or nonspecific proteases; or

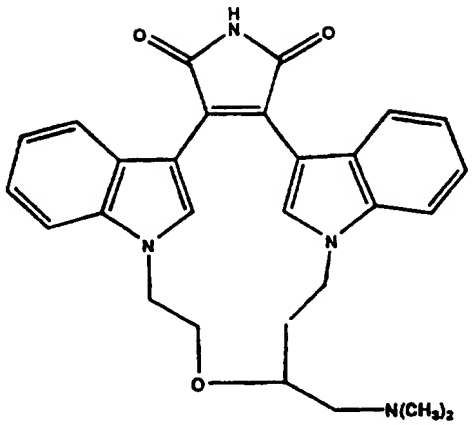
3) derivatives that accumulate at a site of action through membrane selection of a prodrug form or a modified prodrug form; or any combination of 1 to 3, supra. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in H. Bundgaard, Design of Prodrugs, (1985).

The synthesis of various bis-indole-N-maleimide derivatives is described in Davis *et al.* U.S. Patent 5,057,614 and the synthesis of the preferred compounds suitable for use in this invention are described in the previously identified U.S. Patent 5,552,396 and in Faul *et al.* EP publication 0 657 411 A1, all of which are incorporated herein by reference.

One particularly preferred protein kinase C inhibitor for use in the method of this invention is the compound described in Example 5g ((S)-3,4-[N, N'-1,1'-((2"-ethoxy)-3"'(O)-4"'-(N,N-dimethylamino)-butane)-bis-(3,3'-indoly1)]-1(H)-pyrrole-2,5-dione Hydrochloride Salt) of the aforementioned U.S. Patent 5,552,396. This compound is a potent protein kinase C inhibitor. It is selective to protein kinase C over other kinases and is highly isozyme-selective, i.e., it is selective for the beta-1 and beta -2 isozymes. Other salts of this compound also would be favored, especially the mesylate salts.

A preferred mesylate salt can be prepared by reacting a compound of the formula II:

(II)



with methanesulfonic acid in a non-reactive organic solvent, preferably an organic/water mixture, and most preferably water-acetone. Other solvents such as methanol, acetone, ethylacetate and mixtures thereof are operable. The ratio of solvent to water is not critical and generally determined by the solubility of the reagents. Preferred solvent to water ratios are generally from 0.1:1 to 100:1 solvent to water by volume. Preferably, the ratio is 1:1 to 20:1 and most preferably 5:1 to 10:1. The optimal ratio is dependent on the solvent selected and is preferably acetone at a 9:1 solvent to water ratio.

The reaction usually involves approximately equimolar amounts of the two reagents, although other ratios, especially those wherein the methanesulfonic acid is in excess, are operative. The rate of addition of methanesulfonic acid is not critical to the reaction and may be added rapidly (<5 minutes) or slowly over 6 or more hours. The reaction is carried out at temperatures ranging from 0°C to reflux. The reaction mixture is stirred until formation of the salt is complete, as determined by x-ray powder diffraction and can take from 5 minutes to 12 hours.

The salts of the present invention are preferably and readily prepared as a crystalline form. The trihydrate form of the salt may be readily converted to the monohydrate upon drying or exposure to 20-60% relative humidity. The salt is substantially crystalline demonstrating a defined melting point, birefringence, and an x-ray diffraction pattern. Generally, the crystals have less than 10% amorphous solid and preferably less than 5% and most preferably less than 1% amorphous solid.

The mesylate salt is isolated by filtration or other separation techniques appreciated in the art directly from the reaction mixture in yields ranging from 50% to 100%. Recrystallization and other purification techniques known in the art may be used to purify the salt further if desired.

Endothelial cells in tissue culture stimulated by growth factors such as VEGF exhibit a greater growth rate than the basal cellular growth rate. Experiments performed in the present invention have shown that when administered *in vitro*, at a concentration of about 0.1 to 100 nM, the protein kinase C inhibitor, (S)-3,4-[N, N'-1,1'-((2"-ethoxy)-3'"(O)-4'"-(N,N-dimethylamino)-butane)-bis-(3,3'-indoly1)]-1(H)-pyrrole-2,5-dione acid salt, significantly inhibits growth factor (such as VEGF) stimulated non-basal cell growth.

Importantly, other testings have demonstrated that normal endothelial cell growth in tissue culture is not inhibited by this compound, as shown by the lack of inhibition of endothelial cell growth without VEGF stimulation in normoxic conditional media. In hypoxic conditioned media, the cell growth rate increases due to the increase in the content of endogenous growth factor, VEGF, produced by the hypoxic cells. Again, the β-isozyme selective protein kinase C inhibitor (S)-3,4-[N,

N'-1,1'-((2"-ethoxy)-3'"(O)-4'"-(N,N-dimethylamino)-butane)-bis-(3,3'-ind ly1)]-1(H)-pyrrole-2,5-dione acid salt normalizes the cell growth induced by such hypoxic conditions.

Experiments provided in the present invention demonstrate that capillary permeability is also affected by growth factors such as VEGF. Testing has shown that in an animal model, VEGF significantly increases the capillary permeability up to 3 fold. This VEGF dependent capillary permeability increase is also dose dependent. According to the *in vivo* animal testing, administrating protein kinase C inhibitor at a concentration of about 25mg/kg/day prior to VEGF challenge greatly inhibited the capillary permeability induced by VEGF. Use of concentrations from 1nM to 5mM, and preferably from 1nM to 500nM are specifically contemplated. The inhibition can be up to 80% and is generally specific to growth factor induced capillary permeability. Capillary permeability can be measured by fluorescein angiography.

The PKC-β inhibitors of the present invention can be used to treat the disease conditions associated with endothelial cell growth and capillary permeability, especially neoplasia, and other VEGF related diseases.

Pulmonary edema is also treatable by the compounds of the present invention. Pulmonary edema is characterized by increases in the interstitial liquid content of the lungs due to increased capillary permeability. Pulmonary edema can be associated with several disease conditions including Adult Respiratory Distress Syndrome (ARDS). It is likely to be associated primarily with disruption of the alveolar-capillary membranes which could induce hypoxia and subsequent increase in VEGF content. Such disruption could also activate PKC β. Therefore, the compounds

identified in the present invention can interfere with the stimulation of capillary permeability by growth factors and/or PKC β and ameliorate the conditions that lead to pulmonary edema.

The PKC inhibitors of the present invention can also be used to treat neoplasia and other VEGF related diseases in a mammal. The signal transduction pathway of VEGF has direct effects on tumor cells as well as mediates the angiogenic activities in a wide range of neoplastic and non-neoplastic disease conditions. VEGF expression has been demonstrated in a variety of human tumors such as capillary hemangioblastomia, breast cancer, Kaposi's sarcoma, glioblastoma, angiomatous disorders, colorectal cancer, medulloblastoma, gastric carcinoma, adenocarcinomas of the gastrointerestinal tract, malignant melanoma, ovarian cancer, non small cell lung cancer, prostate cancer, bladder carcinoma, von Hippel Lindau Syndrome, renal cell carcinoma, skin cancer, thyroid malignancies, cervical cancer, hepatocellular carcinoma, rhabdomyosarcoma, and leiomysarcoma.

The poor prognosis of a tumor is often associated with the degree of tumor vascularity coupled with VEGF expression. Without a vascular supply, tumor growth is limited. Therefore use of an antiangiogenic agent or anti-VEGF agent may prevent further growth and induce regression of tumors by limiting vascular supply. Anti-VEGF agents may also have direct effects on tumor cells, *e.g.*, VEGF directly affects malignant melanoma cells.

The expression of VEGF is controlled by multiple mechanisms. VEGF production can be positively modulated by hypoxia, certain oncgenea and various cytokines including transforming growth factor-beta (TGF-β), platelet derived growth

In a preferred embodiment, PKC-β inhibitors can be used in anti-VEGF therapy to treat a human with neoplasia. Any neoplastic growth expressing VEGF, *e.g.*, the tumors listed above, can be affected by the PKC-β inhibitors of the present invention. The anti-VEGF therapy is especially preferred to treat a human with unresectable primary tumors, primary tumors that are incompletely removed by surgical or radiotherapeutic techniques, primary tumors which have been adequately treated but who are at high risk to subsequently develop metastatic disease, and those with an established metastatic disease. Groups of tumors having a worse prognosis conferred by a high degree of vascularity *e.g.*, breast cancer, prostate cancer, colon cancer, melanoma cancer, non small cell lung cancer and head/neck carcinoma are especially good candidates for the anti-VEGF therapy or PKC-β inhibitor treatment of the invention.

Hemangioma of infancy occurs in 10-12% of white infants. Generally, it is not a life threatening disorder but in some cases either due to size or anatomic location can cause significant morbidity and mortality. VEGF has been implicated in the growth of these tumors. Currently, interferon α-2a is utilized to induce regression of this tumor. Given the angiogenic nature of this tumor, anti-VEGF therapy employing PKC-β inhibitors should be as efficacious as interferon α-2a or could be assessed as salvage therapy for use upon interferon α-2a failure.

PKC-β inhibitors or anti-VEGF therapy could also be used to treat tumor induced ascites, malignant pleural effusions and peritumoral edema. Given that VEGF is increased in the acitic fluid of females having ovarian hyperstimulation syndrome post induction of ovulation, a PKC-β inhibitor could be of use in this

condition. VEGF is a vascular permeability factor with a high potency, *e.g.*, 50,000 fold greater than histamine. VEGF concentration is elevated in fluid removed from patients with pleural and peritoneal effusions due to malignancy. Intraperitoneal injection of tumor cells into nude mice results in accumulation of ascites that temporally correlates with increasing secretion of VEGF into peritoneum. Peritumoral edema occurring in central nerve system neoplasms such as glioblastoma is associated with high level of VEGF. Anti-VEGF therapy will reduce ascites and pleural effusions associated with malignancy and ovarian hyperstimulation syndrome. Such therapy will decrease the need for repeated paracentesis/thoracentesis and the attendant morbidity associated with these procedures *e.g.*, infection, protein depletion, collapsed lung, etc. Such therapy is especially preferred for inhibition of peritumorial edema occurring in closed anatomic areas, such as central nerve system.

The PKC-β inhibitors used in the present invention can also be used in anti-VEGF therapies to treat other diseases associated with VEGF expression.

Rheumatoid arthritis is characterized by a hyperplastic synovial pannus with a high degree of vascularity which invades and destroys the normal joint architecture. In addition, the exudative nature of synovial fluid suggests a heightened degree of capillary permeability. VEGF can stimulate collagenase expression and further worsen the destructive process. VEGF levels are significantly elevated in synovial fluid derived from patients with rheumatoid arthritis as compared to patients with osteoarthritis. VEGF production has also been localized to infiltrating macrophages. Therefore, rheumatoid arthritis could be treated by administering PKC-β inhibitors in anti-VEGF therapy.

Keloid is characterized by exuberant granulation tissue formation during wound healing that results in hypertrophic scarring. This disorder is typically seen in black patients and tends to be a recurrent disorder. Topical application of PKC inhibitors to hypertrophic granulation tissue could reduce angiogenesis and lessen subsequent scar formation.

Carpal tunnel syndrome, also called entrapment neuropathy, is characterized by compression of nerves which can lead to sensory alternations, muscle weakness, and muscle wasting. It is caused by pressure on the median nerve as it passes through the space formed by the bones of the wrist and the transverse carpal ligament. Carpal tunnel syndrome occurs either as a diabetes related syndrome or in non-diabetic populations.

The enhanced nerve hydration in carpal tunnel syndrome can be caused by elevated level of VEGF. Increased VEGF levels in the nerve surrounding tissues could cause nerve entrapment by inducing vascular permeability and fluid efflux into the perineural tissues. Alteration of synthesis and/or degradation of collagen in carpal tunnel syndrome can be caused by high level of TGF-β production. The increased TGF-β expression could enhance the extracellular protein synthesis including collagen and reduce the degradation thereof which leads to an increased extracellular matrix deposition in the nerve surrounding tissues. PKC activation has been shown to induce the transcription of TGF-β by stimulating activator protein-1 activity. Therefore, PKC-β inhibitors of the present invention can be used to counteract VEGF and/or TGF-β activity in carpal tunnel syndrome.

One skilled in the art will recognize that a therapeutically effective amount of the protein kinase C-β inhibitors used in accordance with the present invention is the amount sufficient to inhibit the growth of endothelial cells or development of capillary permeability by inhibiting VEGF and that this amount varies *inter alia*, depending upon an affected tissue size, the concentration of the compound in the therapeutic formulation, and the body weight of the patient. Generally, an amount of protein kinase C inhibitor to be administered as a therapeutic agent for treating neoplasia and other VEGF related diseases discussed above will be determined on a case by case basis by the attending physician. As a guideline, the extent of the neovascularization, the body weight and age of the patient will be considered when setting an appropriate dose.

Generally, a suitable dose is one that results is a concentration of the protein kinase C inhibitor at the treatment site in the range of 0.5 nM to 200 μM, and more usually 0.5 nM to 200 nM. It is expected that serum concentrations of 0.5 nM to 100 nM should be sufficient in most circumstances.

To obtain these treatment concentrations, a patient in need of treatment likely will be administered between about 0.001 mg per day per kg of body weight and 50.0 mg per day per kg. Usually, not more than about 1.0 to 10.0 mg per day per kg of body weight of protein kinase C-β inhibitor should be needed. As noted above, the above amounts may vary on a case-by-case basis.

The compounds of formula I and the preferred compounds of formula Ia and Ib are preferably formulated prior to administration. Suitable pharmaceutical formulations are prepared by known procedures using well known and readily

available ingredients. In making the compositions suitable for use in the method of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosol (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders for either oral or topical application.

Some examples of suitable carriers, excipient, and diluents include lactose, dextrose, sucrose sorbitol, mannitol, starches, gum acacia, calcium phosphates, alginate, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.05 mg to about 3 g, more usually about 750 mg of the active ingredient. However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the

severity of the condition to be treated, the choice f compound to be administered and the chosen route of administration. Therefore, the above dosage ranges are not intended to limit the scope of the invention in any way. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

In addition to the above formulations, most of which may be administered orally, the compounds used in the method of the present invention also may be administered topically. Topical formulations include ointments, creams and gels.

Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petrolatum or mineral oil, or (2) an absorbent base, i.e., one consisting of an anhydrous substance or substances which can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the active ingredient (compound) is added to an amount affording the desired concentration.

Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons, and the like, such as waxes, petrolatum, mineral oil, and the like, and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilized by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfate; hydrophilic colloids, such as acacia colloidal clays, veegum, and the

like. Upon formation of the emulsion, the active ingredient (compound) customarily is added in an amount to achieve the desired concentration.

Gels comprise a base selected from an oleaginous base, water, or an emulsion-suspension base. To the base is added a gelling agent which forms a matrix in the base, increasing its viscosity. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers, and the like. Customarily, the active ingredient (compounds) is added to the formulation at the desired concentration at a point preceding addition of the gelling agent.

The amount of compound incorporated into a topical formulation is not critical; the concentration should be within a range sufficient to permit ready application of the formulation to the affected tissue area in an amount which will deliver the desired amount of compound to the desired treatment site.

The customary amount of a topical formulation to be applied to an affected tissue will depend upon an affected tissue size and concentration of compound in the formulation. Generally, the formulation will be applied to the effected tissue in an amount affording from about 1 to about 500 μg compound per cm^2 of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300 $\mu g/cm^2$, more preferably, from about 50 to about 200 $\mu g/cm^2$, and, most preferably, from about 60 to about 100 $\mu g/cm^2$.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active agent	250
starch, dried	200
magnesium stearate	10
Total	460 mg

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

Formulation 2

A tablet is prepared using the ingredients below:

	Quantity (mg/capsule)
Active agent	250
cellulose, microcrystalline	400
silicon dioxide, fumed	10
stearic acid	5
Total	665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

Tablets each containing 60 mg of active ingredient are made as follows:

	Quantity (mg/tablet)
Active agent	60 mg
starch	45 mg
microcrystalline cellulose	35 mg
polyvinylpyrrolidone (as 10% solution in water)	4 mg
sodium carboxymethyl starch	4.5 mg
magnesium stearate	0.5 mg
talc	1 mg
Total	150 mg

The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

EXAMPLES

These examples all demonstrate the use of (S)-3,4-[N, N'-1,1'-((2"-ethoxy)-3'"(O)-4'"-(N,N-dimethylamino)-butane)-bis-(3,3'-indoly1)]-1(H)-pyrrole-2,5-dione hydrochloride salt to inhibit *in vitro* endothelial cell growth and *in vivo* increased capillary permeability stimulated by VEGF .

Example 1

In this example, the inhibitory effect of the noted compound on VEGF stimulated endothelial cell growth was examined using recombinant human VEGF.

Bovine retinal endothelial cells were isolated from fresh calf eyes by homogenization and a series of filtration steps. Primary endothelial cell cultures were grown in fibronectin (NYBen Reagents, New York Blood Center)-coated dishes (Costar) containing Dulbecco's modified Eagle's medium (DMEM) with 5.5 mM glucose, 10% plasma-derived horse serum (Wheaton, Scientific). 50 mg of heparin per liter and 50 units of endothelial cell growth factor per liter (Boehringer Mannheim). After the cells reached confluence, the medium was changed to include 5% fetal bovine scrum (HyClonc). Medium was changed every 3 days. Endothelial cell homogeneity was confirmed with anti-factor VIII antibodies.

The effect of the noted PKC inhibitor on VEGF action *in vitro* was evaluated by using sparsely plated cultures of the bovine retinal microvascular endothelial cells, which undergo growth stimulation upon addition of VEGF. Bovine retinal endothelial cells were plated sparsely (~2500 cells per well) in 24-well dishes (Costar), incubated overnight in DMEM containing 10% calf serum (GIBCO). The medium was changed the next day.

To examine the impact of the noted PKC inhibitor on endothelial cell growth, one set of experiments was conducted in which the cell growth in the absence of any active agent served as a control, and then the impact of the addition of the noted PKC inhibitor in both the presence of VEGF (25 ng/ml; Genentech) and in the absence of VEGF was examined. After incubation at 37°C for 4 days, the cells were lysed in

0.1% sodium dodecyl sulfate (SDS) and DNA content was measured using H echst 33258 dye and a fluorometer (model TKO-100; Hoefer).

All determinations were performed at least in triplicate and experiments were repeated a minimum of three times. Results are expressed as means ± SD for all experiments. Analysis of *in vitro* results was performed by non-paired Student's *t* test. A *P* value of <0.050 was considered statistically significant.

Figure 1 illustrates the results obtained using recombinant VEGF. As shown by the three left-most columns of the figure, the addition of the noted PKC inhibitor to the endothelial cell culture had essentially no impact on the basal growth rate (column one). The growth rate increased substantially upon the addition of VEGF (fourth column). This growth rate was curtailed significantly upon the addition of >0.5nM of the noted PKC inhibitor (four right-most columns).

Example 2

This example is similar to the work reported in Figure 1 and further illustrates the inhibitory effect of the noted PKC inhibitor on VEGF stimulated endothelial cell growth using recombinant human VEGF.

Using the procedures of Example 1, bovine retinal endothelial cells were isolated and grown; then sparsely plated cultures were prepared. Again, using the procedure of Example 1, experiments were conducted in which the affect of the noted PKC inhibitor on endothelial cell growth in both the presence of VEGF (25 ng/ml; Genentech) and in the absence of VEGF was examined. After incubation at 37°C for 4 days, the cells were lysed in 0.1% sodium dodecyl sulfate (SDS) and DNA content was measured using Hoechst 33258 dye and a fluorometer (model TKO-100; Hoefer).

Figure 2 illustrates the results of this work. As shown by the columns above the legend -VEGF, the addition of the noted PKC inhibitor to the endothelial cell culture at from 0.1 nM to 100 nM had essentially no impact on the basal growth rate of the cells. Stimulation of the endothelial cells with recombinant human VEGF (25 ng/ml) produced a significant increase in cellular DNA content after 4 days, indicative of an increase in growth rate, compared with unstimulated cells (compare -VEGF at 0 with +VEGF at 0). This growth rate was curtailed significantly upon the addition of the noted PKC inhibitor (four right-most columns above legend +VEGF). In particular, the VEGF stimulatory capacity was reduced slightly in the presence of 0.1 nM of the PKC inhibitor and was essentially entirely eliminated by simultaneous addition of 1 nM and greater of the PKC inhibitor.

Example 3

This example examines the impact of the noted PKC inhibitor on the activity of endogenous VEGF expressed upon culturing retinal pericytes under hypoxic conditions.

Bovine retinal endothelial cells and retinal pericytes were isolated from fresh calf eyes by homogenization and a series of filtration steps. The endothelial cells were grown and sparsely cultured on plates using the procedures of Example 1. Using similar techniques, bovine retinal pericytes were cultured in DMEM/5.5 mM glucose with 20% fetal bovine serum.

Hypoxic conditioned medium for endogenous VEGF expression and normoxic conditioned control medium were prepared respectively according to the following procedures. Confluent retinal pericyte monolayers were exposed for 24 hr to 2%

0_2/5% CO_2/93% N_2 using a Lab-Line Instruments advanced computer controlled infrared water-jacketed CO_2 incubator with reduced oxygen control (model 480). All cells were maintained at 37°C and showed no morphologic changes by light microscopy, excluded trypan blue dye (>98%) and could subsequently be passaged normally. Cells incubated under normoxic conditions (95% air/5% CO_2) from the same batch and passage were used as controls. Medium was subsequently collected and filtered (Nalgene; 0.22μm) prior to use.

In this example, experiments were conducted in which the affect of the noted PKC inhibitor on endothelial cell growth in the presence of either normoxic conditioned media or hypoxic conditioned media was examined. As was done in the previous examples, after incubation at 37°C for 4 days, the cells were lysed in 0.1% sodium dodecyl sulfate (SDS) and DNA content was measured using Hoechst 33258 dye and a fluorometer (model TKO-100; Hoefer).

In the tests reported in Figure 3, the noted PKC inhibitor was used at a concentration of 10 nM. As shown in Figure 3, retinal endothelial cell growth was stimulated by conditioned medium from retinal pericytes cultured under hypoxic conditions known to induce VEGF expression (compare column 1 to column 3 in Figure 3). This growth stimulation was suppressed (normalized) in the presence of the hydrochloric acid salt of (S)-3,4-[N, N'-1,1'-((2"-ethoxy)-3"'(O)-4"'-(N,N-dimethylamino)-butane)-bis-(3,3'-indoly1)]-1(H)-pyrrole-2,5-dione of the PKC inhibitor (compare 3 to column 4).

Example 4

This example is similar to the work reported in Figures 1 and 2 and further illustrates the inhibitory effect of the noted PKC inhibitor on VEGF stimulated endothelial cell growth using recombinant human VEGF.

Using the procedures of Example 1, bovine retinal endothelial cells were isolated and grown; then sparsely plated cultures were prepared. Again, using the procedure of Example 1, experiments were conducted in which the affect of the noted PKC inhibitor on endothelial cell growth in both the presence (+VEGF)(25 ng/ml; Genentech) and absence of VEGF (-VEGF) were examined. As above, after incubation at 37°C for 4 days, the cells were lysed in 0.1% sodium dodecyl sulfate (SDS) and DNA content was measured using Hoechst 33258 dye and a fluorometer (model TKO-100; Hoefer).

Figure 4 illustrates the results of this work. As shown by the columns above the legend -VEGF, the addition of the noted PKC inhibitor to the endothelial cell culture at a concentration of 10 nM had essentially no impact on the basal growth rate of the cells. Stimulation of the endothelial cells with recombinant human VEGF (25 ng/ml) produced a significant increase in cellular DNA content, indicative of an increase in growth rate, compared with unstimulated cells (compare -VEGF Control with +VEGF Control). This growth rate was curtailed significantly upon the addition of the noted PKC inhibitor at a concentration of 10 nM.

These results demonstrate that the disclosed class of PKC inhibitors and particularly, (S)-3,4-[N,N'-1,1'-((2"-ethoxy)-3'"(O)-4'"-(N,N-dimethylamino)-butane)-bis-(3,3'-indoly1)]-1(H)-pyrrole-2,5-dione, prevents *in vitro* stimulation of retinal

endothelial cell growth by both exogenous and hypoxia-induced VEGF. Since VEGF expression has been linked closely with neovascularization associated with macular degeneration, these results support the use of these PKC inhibitors as a therapy for the treatment of macular degeneration.

The principles, preferred embodiments and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed, since they are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in the art without departing from the spirit of the invention.

CLAIMS:

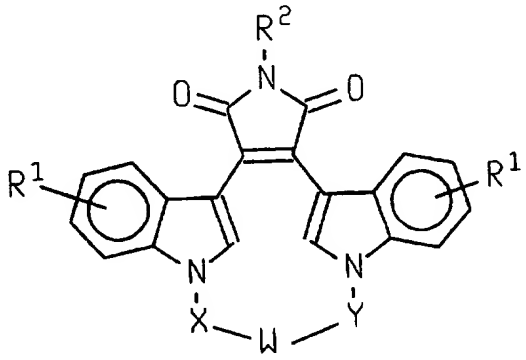
1. A method for treating a neoplasia which comprises administering to a mammal in need of such treatment, a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

2. The method of claim 1 wherein the inhibitor of the β isozyme of protein kinase C is a bis-indolylmaleimide or a macrocyclic bis-indolylmaleimide.

3. The method of claim 1 wherein the inhibitor is isozyme selective and where the isozyme selectivity is selected from the group consisting of beta-1 and beta-2 isozymes.

4. The method of claim 3 wherein the protein kinase C inhibitor has the following formula:

(I)



wherein:

W is -O-, -S-, -SO-, -SO_2-, -CO-, C_2-C_6 alkylene, substituted alkylene, C_2-C_6 alkenylene, -aryl-, -aryl$(CH_2)_m$O-, -heterocycle-, -heterocycle-$(CH_2)_m$O-, -fused bicyclic-, -fused bicyclic-$(CH_2)_m$O-, -NR^3-, -NOR^3-, -CONH-, or -NHCO-;

X and Y are independently C_1-C_4 alkylene, substituted alkylene, or together X, Y, and W combine to form -$(CH_2)_n$-AA-;

R^1s are hydrogen or up to four optional substituents independently selected from halo, C_1-C_4 alkyl, hydroxy, C_1-C_4 alkoxy, haloalkyl, nitro, NR^4R^5, or -NHCO(C_1-C_4 alkyl);

R^2 is hydrogen, CH_3CO-, NH_2, or hydroxy;

R^3 is hydrogen, $(CH_2)_m$aryl, C_1-C_4 alkyl, -COO(C_1-C_4 alkyl), -$CONR^4R^5$, -(C=NH)NH_2, -SO(C_1-C_4 alkyl), -SO_2 (NR^4R^5), or -SO_2 (C_1-C_4 alkyl);

R^4 and R^5 are independently hydrogen, C_1-C_4 alkyl, phenyl, benzyl, or combine to the nitrogen to which they are bonded to form a saturated or unsaturated 5 or 6 member ring;

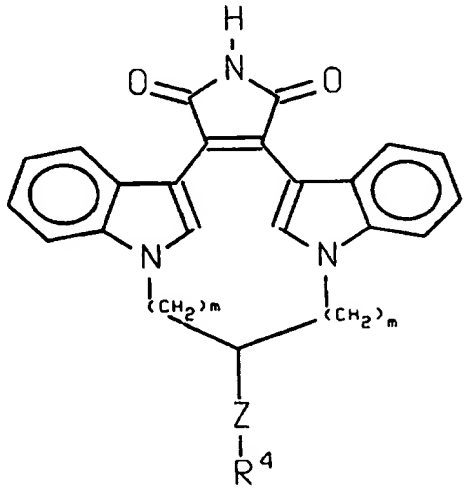
AA is an amino acid residue;

m is independently 0, 1, 2, or 3; and

n is independently 2, 3, 4, or 5

or a pharmaceutically acceptable salt, prodrug or ester thereof.

5. The method of claim 4 wherein the protein kinase C inhibitor has the following formula:

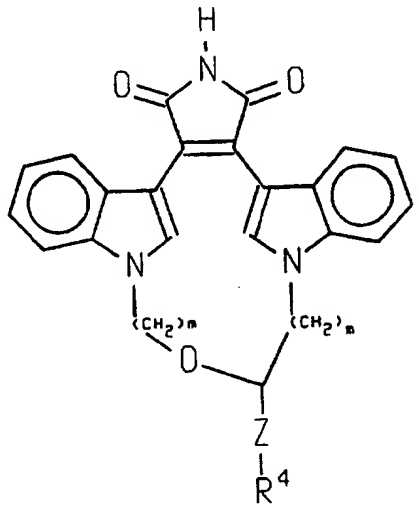


(Ia)

wherein Z is $-(CH_2)_p-$ or $-(CH_2)_p-O-(CH_2)_p-$; R^4 is hydroxy, -SH, C_1-C_4 alkyl, $(CH_2)_m$aryl, -NH(aryl), $-N(CH_3)(CF_3)$, $-NH(CF_3)$, or $-NR^5R^6$; R^5 is hydrogen or C_1-C_4 alky; R^6 is or a pharmaceutically acceptable salt, prodrug or ester thereof.

6. The method of claim 4 wherein the protein kinase C inhibitor has the following formula:

(Ib)



wherein Z is $-(CH_2)_p-$; R^4 is $-NR^5R^6$, $-NH(CF_3)$, or $-N(CH_3)(CF_3)$; R^5 and R^6 are independently H or C_1-C_4 alkyl; p is 0, 1, or 2; and m is independently 2 or 3, or a pharmaceutically acceptable salt, prodrug or ester thereof.

7. The method of claim 5, wherein the protein kinase C inhibitor comprises (S)-3,4-[N, N'-1,1'-((2"-ethoxy)-3'"(O)-4'"-(N,N-dimethylamino)-butane)-bis-(3,3'-indoly1)]-1(H)-pyrrole-2,5-dione or its pharmaceutically acceptable acid salt.

8. The method of claim 1 wherein the neoplasia is selected from the group consisting of capillary hemangioblastomia, breast cancer, Kaposi's sarcoma, glioblastoma, angiomatous disorders, hemangioma of infancy, colorectal cancer, medulloblastoma, gastric carcinoma, adenocarcinomas of the gastrointerestinal tract,

malignant melanoma, ovarian cancer, non small cell lung cancer, prostate cancer, malignant effusions, preitumoral edema, bladder carcinoma, von Hippel Lindau Syndrome, renal cell carcinoma, skin cancer, thyroid malignancies, cervical cancer, hepatocellular carcinoma, rhabdomyosarcoma, and leiomysarcoma.

9. The method of claim 10 wherein the neoplasia is selected from the group consisting of capillary hemangioblastomia, breast cancer, Kaposi's sarcoma, glioblastoma, angiomatous disorders, hemangioma of infancy, colorectal cancer, malignant melanoma, ovarian cancer, non small cell lung cancer, prostate cancer, malignant effusions, preitumoral edema, bladder carcinoma.

10. A method for treating rheumatoid arthritis, which comprises administering to a mammal in need of such treatment, a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

11. A method for treating pulmonary edema, which comprises administering to a mammal in need of such treatment, a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

12. A method for inhibiting VEGF stimulated capillary permeability associated with pulmonary edema, which comprises administering to a mammal in need of such treatment, a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

13. A method for treating keloid, which comprises administering to a mammal in need of such treatment, a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

14. A method for treating Carpal tunnel syndrome, which comprises administering to a mammal in need of such treatment, a therapeutically effective amount of an inhibitor of the β isozyme of protein kinase C.

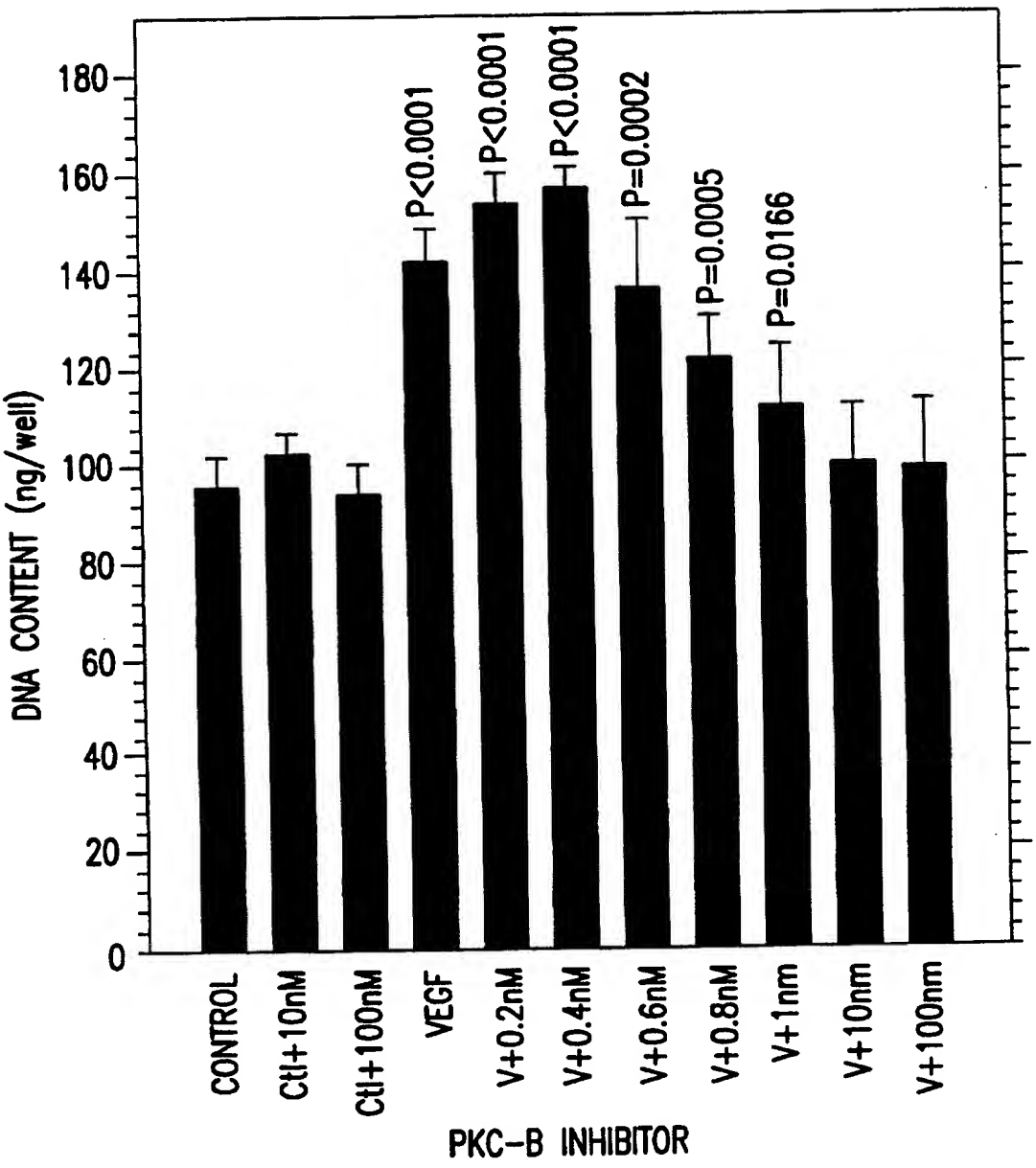

DNA CONTENT (ng/well)
0
20
40
60
80
100
120
140
160
180
P<0.0001
P<0.0001
P<0.0001
P=0.0002
P=0.0005
P=0.0166
CONTROL
Ctl+10nM
Ctl+100nM
VEGF
V+0.2nM
V+0.4nM
V+0.6nM
V+0.8nM
V+1nm
V+10nm
V+100nm
PKC-B INHIBITOR

FIG.1

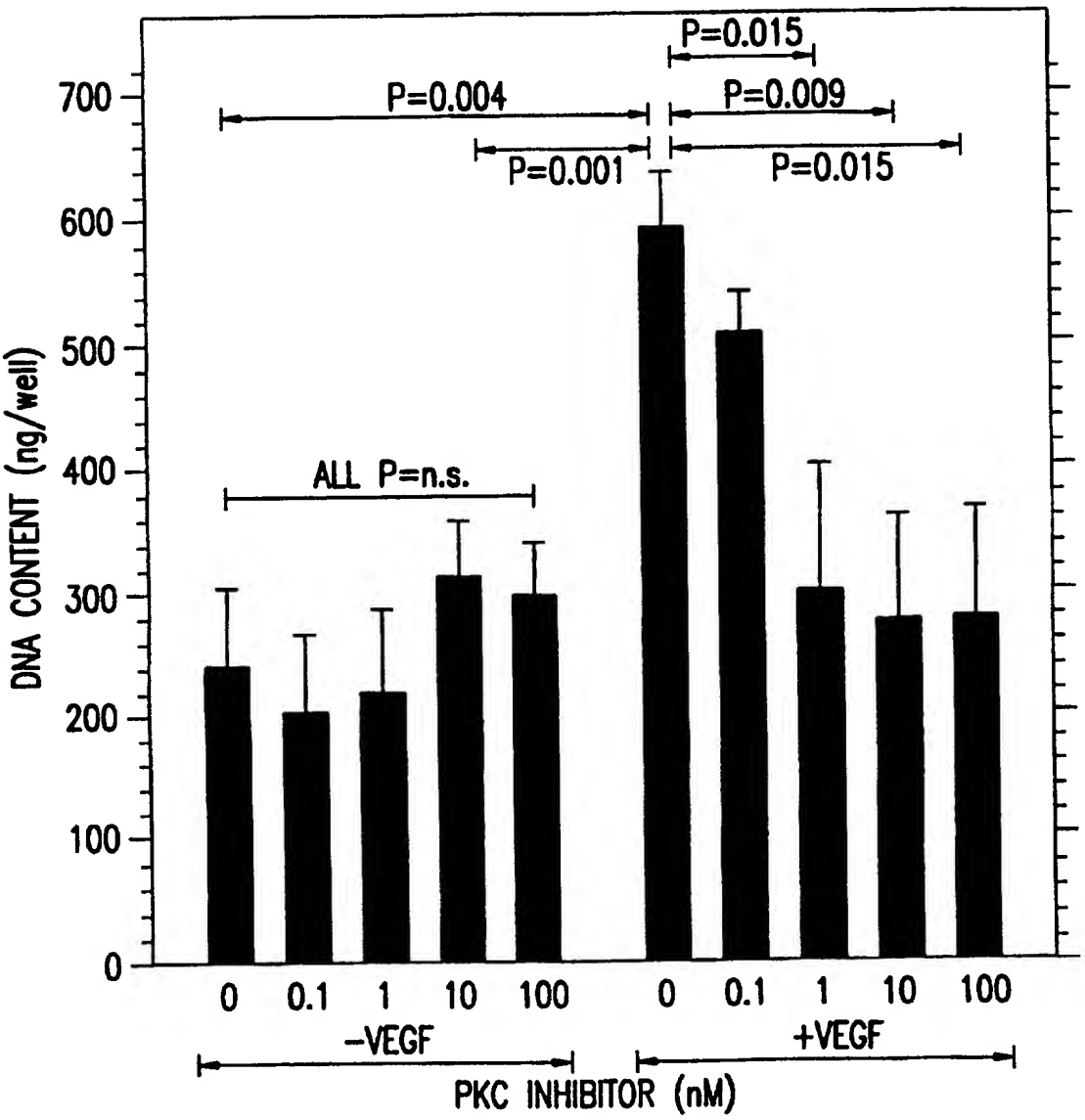

P=0.015
P=0.004
P=0.009
P=0.001
P=0.015
ALL P=n.s.
DNA CONTENT (ng/well)
700
600
500
400
300
200
100
0
0 0.1 1 10 100
0 0.1 1 10 100
-VEGF
+VEGF
PKC INHIBITOR (nM)

FIG.2

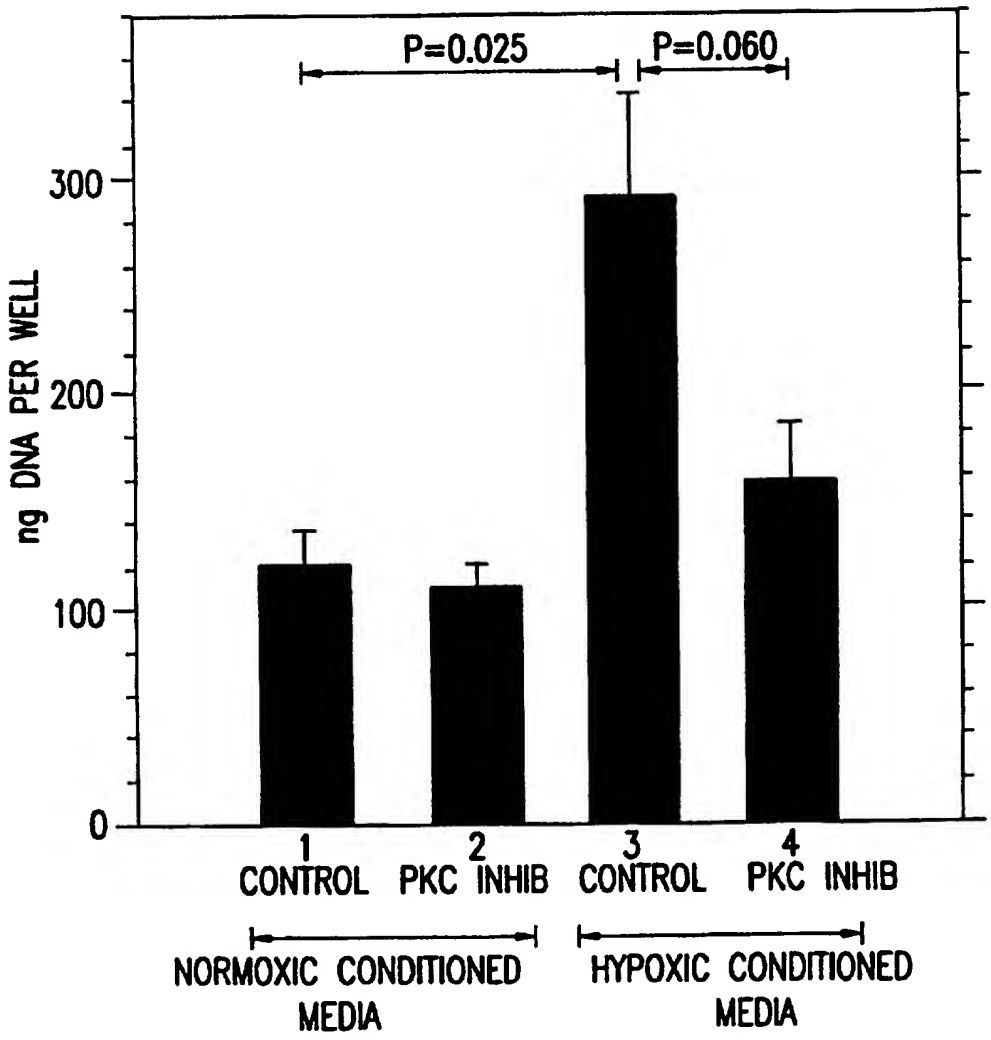

P=0.025
P=0.060
300
200
100
0
ng DNA PER WELL
1
CONTROL
2
PKC INHIB
3
CONTROL
4
PKC INHIB
NORMOXIC CONDITIONED MEDIA
HYPOXIC CONDITIONED MEDIA

FIG.3

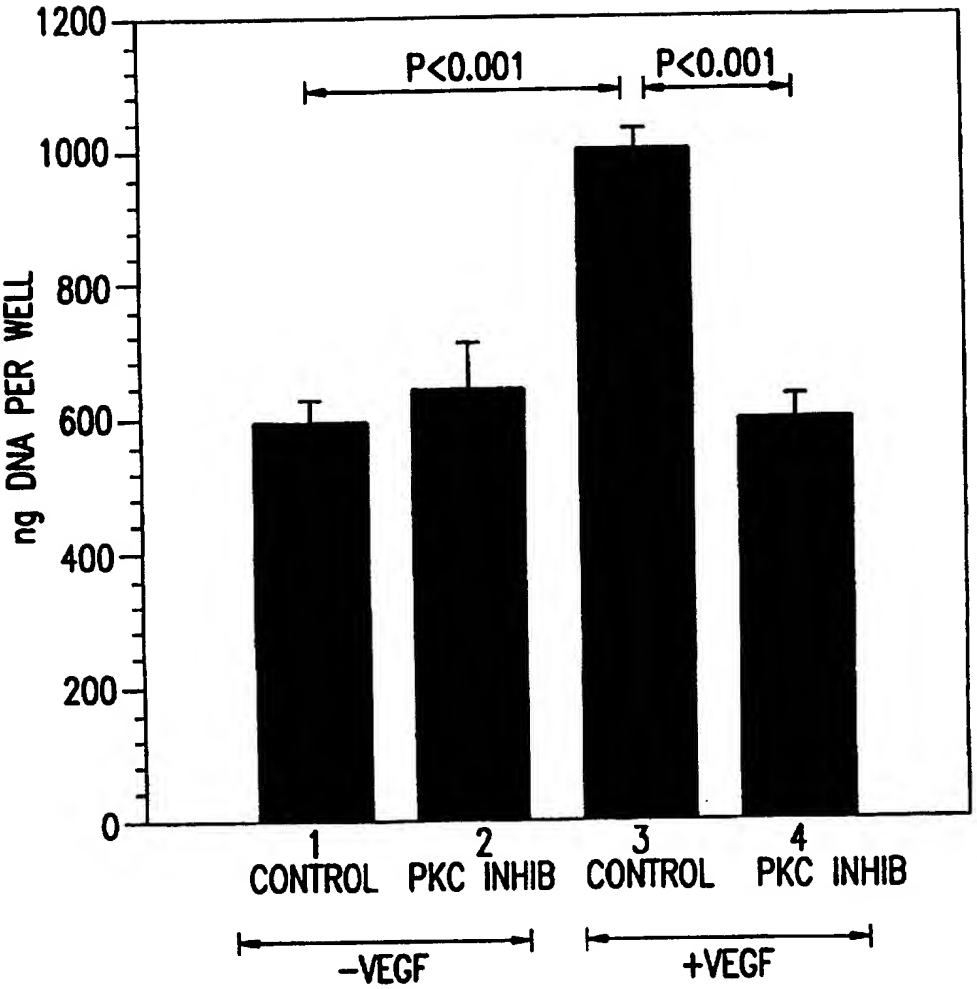

1200
1000
800
600
400
200
0
ng DNA PER WELL
P<0.001
P<0.001
1
CONTROL
2
PKC INHIB
3
CONTROL
4
PKC INHIB
-VEGF
+VEGF

FIG.4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07752

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/40
US CL :514/411

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/411

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	US 5,552,396 A (HEATH, JR. ET AL.) 03 September 1996, see entire document.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 17 JULY 1997	Date of mailing of the international search report 21 AUG 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEROME M. GOLDBERG Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*